TECAN.

Application guide for Tecan plate washers

Your easy way to optimized results

☑ 21 CFR Part 11*

☑ 98/79/EC IVD-D*

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HydroFlex strip washer for 96-well plates

The HydroFlex microplate washer is a truly flexible platform providing excellent automated microplate strip washing and vacuum filtration for 96-well microplate formats. Depending on the instrument configuration, it is capable of processing eight or sixteen wells in parallel, making it ideal for low and medium throughput needs.

This modular and upgradeable platform is ideal for a wide range of cell-, enzyme- and DNA-based applications, especially in academia and clinical diagnostics, but also in biotech and pharma, reflecting over 30 years of Tecan expertise in advanced liquid handling.

The multifunctional HydroFlex microplate washer offers far greater flexibility than a standard microplate washer, and features user-interchangeable plate carriers for easy switching between washing and filtration applications.

The HydroFlex features an ELISA configuration designed to meet the 98/79/EC IVD directive* for in vitro diagnostic products and, in combination with HydroControl™ software, it is prepared to meet the FDA 21 CFR part 11* regulation.

To watch a video of the HydroFlex, visit:
www.tecan.com/hydroflex

Product highlights at a glance

- Ready to run for ELISA washing and gentle cell washing in 96-well plates
- HydroFlex is upgradeable on site to include a magnetic carrier with a patent-pending design using two magnets per well
- Optional vacuum filtration units for processing;
  - non-magnetic beads via filter-bottom plates
  - DNA-purification to remove salts and oligos after a PCR step.
- Multipoint aspiration capability for 96-well flat-bottom plates, to minimize residual volumes per well
- Fast washing of entire wells to minimize assay background signals, helping to remove traces of reagents remaining at the top of the wells from pipetting steps
- Automated buffer switching within a wash protocol included as standard
- Advanced process controls for safety and reliability, including an air bubble sensor in the dispense system to detect when wash buffer bottles are empty
- Easy operation via a library of predefined plate settings
- Integrated keypad and display for on-board operation without an external computer
- IQ/OQ procedure available from Tecan.

Tecan's microplate washers – an overview

The HydroSpeed washer for fast washing of 96- and 384-well plates

HydroSpeed plate washer for 96- and 384-well plates

The HydroSpeed is Tecan's advanced microplate washer, designed to provide optimized washing of cells, beads and ELISAs in both 96- and 384-well formats. It offers full control over critical wash parameters to help maximize the productivity and consistency of your assays.

Depending on the wash head configuration, the HydroSpeed is capable of washing 96 or 384 wells simultaneously, making it ideal for higher throughput needs.

Based on more than 30 years of experience in automated liquid handling, Tecan understands that advanced plate washing applications require precise control of dispensing and aspiration settings to avoid loss of material and poor reproducibility.

With full on-board operation via the easy-to-use touchscreen interface, the HydroSpeed combines power and control in one instrument for optimized washing of a range of cell-, bead- and ELISA-applications in biotech, pharma and academia, as well as in clinical diagnostics.

The HydroSpeed configured for ELISA washing is designed to meet the 98/79/EC IVD directive* for in vitro diagnostic products and, in combination with HydroControl software, it is prepared to meet the FDA 21 CFR part 11* regulation.

To watch video sequences of the HydroSpeed plate washer, visit:
www.tecan.com/hydrospeed
www.tecan.com/cell-protection

Product highlights at a glance

- Cell, bead and ELISA washing in 96- and 384-well plate formats
- Large touchscreen user interface for easy on-board operation in five languages
- Predefined plate library and innovative plate assistant function for easy plate teaching of new plates
- Cell Protection™ wash settings including extra gentle aspiration and dispensing for high cell retention and avoiding damage to the cell layer
- Ideal for processing loosely adherent cells, such as P-815 cells
- Innovative Anti-Clogging™ function prevents needle blockage by automated rinsing and soaking of the wash head
- Wash head design allows intense needle cleaning in a typical ultrasonic bath
- Upgradeable on site with magnetic carriers for efficient washing of a range of magnetic beads
- Optional vacuum filtration module for processing of non-magnetic beads via filter-bottom plates in 96- and 384-well formats
- Maintenance-free liquid level detection for the waste bottle, eliminating the need to clean sensors which are typically in contact with potentially hazardous waste liquid
- Automated buffer switching within a wash protocol included as standard
- Multipoint aspiration to minimize the residual volume in 96-well flat-bottom plates
- Ready for integration onto Tecan robotic platforms
- IQ/OQ procedure available from Tecan.

Tecan's microplate washers – an overview

Washer comparison

Washer:	HydroFlex™	HydroSpeed™
Versatile strip washer	x	
High performance plate washer		x
Plate formats:		
Full microplates, 96-well format	x	x
Full microplates, 384-well format		x
Strip microplates, 96-well format	x	
Wash characteristics & plate formats:		
96-well plates: 8 wells washed in parallel	x	
96-well plates: 16 wells washed in parallel	x	
96-well plates: fast, parallel wash of entire plate		x
384-well plates: fast, parallel wash of entire plate		x
Optimized for high flexibility:		
One wash head (96i) for ELISA, beads & cells in 96- & 384-well format		x
User interfaces:		
Touchscreen interface for onboard operation		x
Display/keypad for onboard operation	x	
HydroControl™ software for operation via external PC	x	x
Ease-of-use:		
Pre-defined plate library	x	x
Pre-defined example programs	x	x
Designed for reliable operation:		
Integrated Anti-Clogging™ function		x
External ultrasonic cleaning of wash heads	x	x
Pre-defined rinse procedure for easy maintenance	x	x
Key applications:		
Gentle cell washing	x	x
Magnetic bead washing: mutiplexed assays	x	x
Vacuum filtration: filter washing of non-magnetic beads	x	x
ELISA washing	x	x
Ready for automation:		
Connect™ plate stacker for 30 / 50 plates per run		x
Tecan Freedom EVOlyzer®: ELISA processor	x	
Tecan Freedom EVO®	x	x
Designed to meet regulatory needs:		
98/79/EC IVD-D using ELISA configuration	x	x
21 CFR part 11 using HydroControl software	x	x
Class 1 general purpose device for US and Canada	x	x

Microplate washer applications at a glance

HydroSpeed plate washer supports these applications in 96-well and 384-well plate formats. HydroFlex strip washer supports these applications in 96-well plate format.

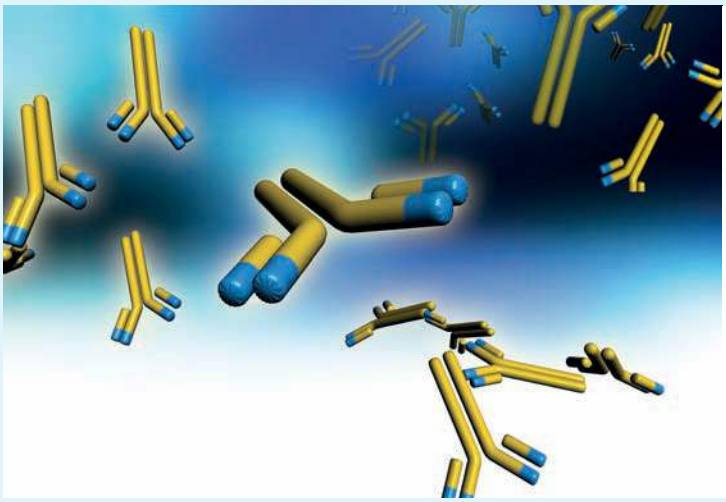
For many years, microplate washers were mainly used for typical ELISA applications. Following innovations in this area, such as various bead-based technologies for multiplexed ELISA assays and the dramatic increase of cell-based applications in the last couple of years, plate washers are now used far more widely.

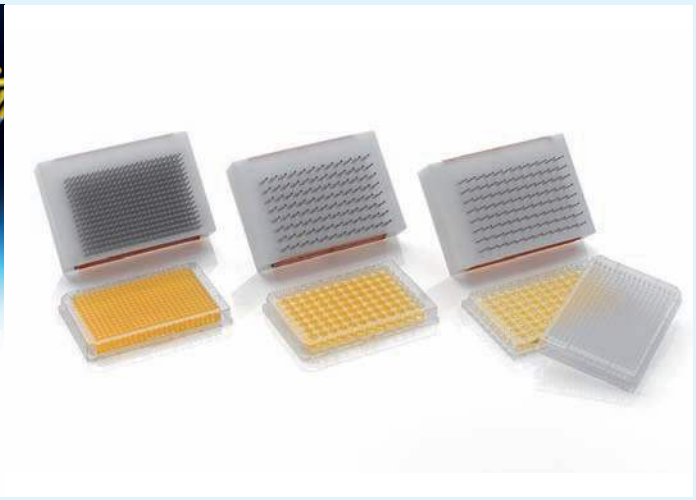
Tecan has developed the HydroFlex and the HydroSpeed as multifunctional plate washers to address these new developments, combining four application solutions into one compact instrument. Tecan washers are now frequently used for the preparation steps of a range of new applications, such as gentle washing of adherent or weakly adherent cells, washing magnetic beads from Luminex and other suppliers, vacuum filtration using filter-bottom plates for processing non-magnetic beads, and DNA purification after PCR.

The HydroFlex and the HydroSpeed are equipped with innovative functions providing full control over critical wash parameters, helping to optimize the retention of analytes during all of the wash steps and resulting in improved consistency and productivity for your assays.

Additionally, Tecan plate washers have been designed for reliable operation and ease-of-use, including predefined maintenance programs to prevent clogging of the wash head, as well as ready-to-use plate libraries for getting started easily.

ELISA washing





Wash heads available for Tecan's HydroSpeed plate washer (from left to right): high speed 384-channel wash head (384HT), dedicated 96-channel wash head (96HT) and universal 96-indexing wash head (96i)

Principle

Enzyme-linked immunosorbent assays (ELISAs) use antibody recognition and a subsequent absorbance-, fluorescescence- or luminescence-based signal to identify (and quantify) a substance.

Typically, an unknown amount of a particular antigen (sample) is immobilized to a surface, such as the wells of a polystyrene microplate. This binding event is either via non-specific adsorption of the antigen to the surface (direct or indirect ELISAs, see figure 1), or by highly specific capture of the antigen on an immobilized antibody ('sandwich' ELISA, figure 2).

After the antigen has been immobilized onto the surface of the wells via specific or non-specific binding, the detection antibody is added, forming a complex with the antigen. This detection antibody can be directly linked to an enzyme – which will trigger the subsequent detection signal – or it can be linked indirectly to a secondary antibody, which is connected to an enzyme through bioconjugation.

Between each step of the schematic workflow, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies which have not bound to the wells (see figures 1-2). During the wash steps, only the antigen and its specific binding counterparts remain bound to the surface of the wells via antigen-antibody interactions, while the non-specific or unbound components of the samples are washed away.

In a final reaction, the enzyme connected to the detection antibody consumes a corresponding substrate to produce a detectable signal. Most commonly, this results in a color change in the wells (absorbance-based ELISAs), which is proportional to the quantity of antigen in the sample.
There are also fluorescence- and luminescence-based ELISAs on the market, which are generally more sensitive than absorbance-based ELISAs.

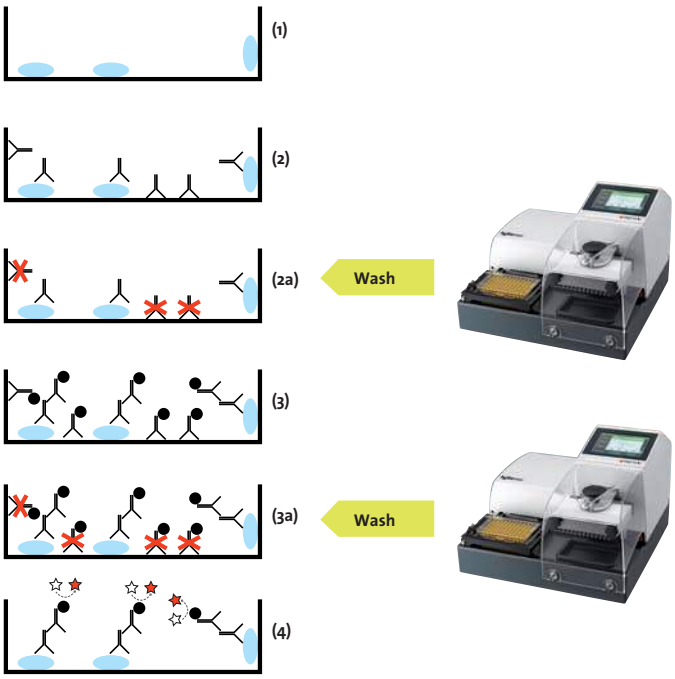


Figure 1: Indirect ELISA: (1) Plate is coated with an antigen (sample); (2) primary antibody is added, and any antigen present binds to this antibody; (3) enzyme-linked secondary antibody is added and binds to the primary antibody; (4) substrate is added, and converted by the antibody-bound enzyme to a detectable form. Between each step the plate is washed with a wash buffer (mild detergent, often containing high salt concentrations).

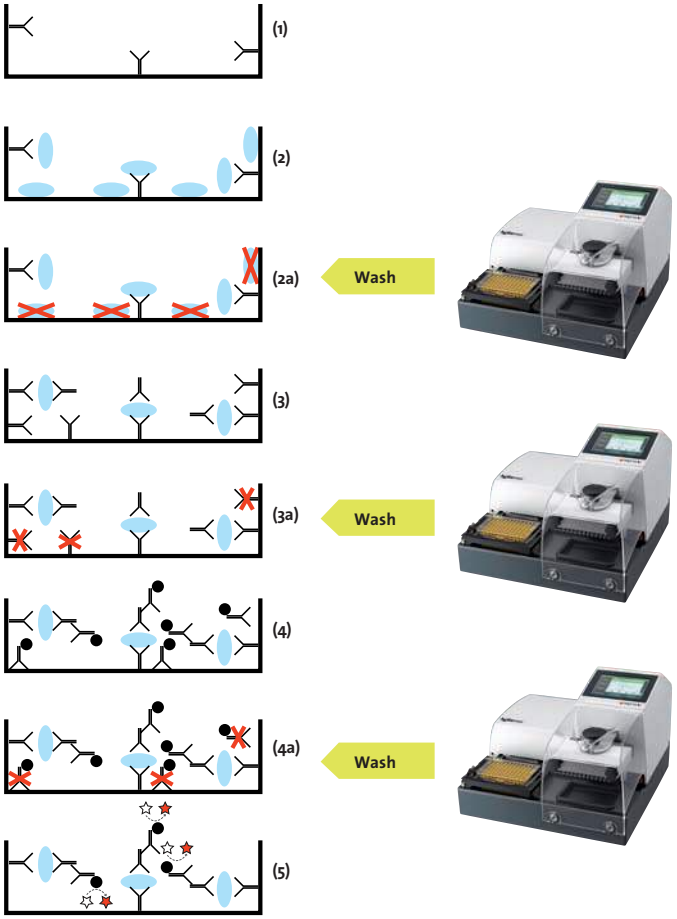


Figure 2: Sandwich ELISA: (1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detection antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by the secondary antibody-bound enzyme to detectable form. Between each step the plate is washed with a wash buffer (mild detergent, often containing high salt concentrations)

Critical parameters

The wash steps are perhaps the most critical elements in the whole ELISA procedure. Besides the fact that the washing buffers need to be optimized from a chemical perspective, the type of wash procedure chosen also critically influences the final results.

Manual washing of ELISA plates is common, but this approach is not only tedious, but also susceptible to individual, user-specific variation and error which may lead to low overall reproducibility between operators. Using a microplate washer helps to increase throughput, reproducibility and comparability of ELISA applications, as well as helps saving time and money.
When selecting a microplate washer for processing ELISA plates, a number of aspects should be considered. In the majority of commercial ELISAs, the specific and non-specific binding of antibodies is quite strong, and so vigorous wash conditions are typically required, such as high speed dispensing and aspirating. However, there are also custom-made ELISAs available where the specific binding to the surface of the wells is quite weak, making it necessary to use gentle washing conditions.

Tecan solution

Wash conditions in Tecan plate washers can be finely adjusted to meet individual assays requirements. Good washing efficiency relies on minimizing the residual volume in the wells after washing. This is especially challenging in 96-well flat bottom plates, due to the large diameter and overall geometry of the wells. Tecan plate washers overcome this issue by aspirating from up to four different points in the plate.

Similarly, small residues of reagents may sometimes be left at the top of wells, typically from manual multichannel pipetting. Non-specific binding of antibodies takes place across the whole well, and so this can lead to an elevated background signal. To avoid this, Tecan plate washers can wash the entire surface of the wells, using a wash volume that is typically twice that of the well. This is achieved using an ‘overflow wash mode’ which simultaneously dispenses and aspirates the wash buffer.

Microplate washers used for ELISAs are often susceptible to needle clogging during idle-time between runs, because the washing buffers typically contain high salt concentrations and are therefore prone to crystallization.

To prevent this, Tecan washers offer pre-defined rinse procedures for easy maintenance, and feature wash heads that can be easily removed for thorough cleaning in an ultrasonic bath. In addition, the HydroSpeed plate washer is equipped with a unique Anti-Clogging function which actively prevents needle blockage by automatically rinsing and soaking the wash head when the washer is waiting idle between plates.

Washer comparison for ELISA applications

Washer:	HydroFlex™	HydroSpeed™
96-well plates	x	x
384-well plates		x
96-well strip plates	x	
Aspiration fine tuning	x	x
Dispensing fine tuning	x	x
Overflow washing	x	x
External ultrasonic cleaning of wash heads	x	x
Multiple aspiration points for minimized residual volume	x	x
Predefined rinse procedure for easy maintenance	x	x
Integrated Anti-Clogging		x
Designed to meet the IVD-D 98/79/EC	x	x
Prepared to meet 21 CFR part 11 in combination with HydroControl™ software	x	x

Typical ELISA wash programs for the 96-well plate format using a HydroFlex or HydroSpeed plate washer

	HydroFlex™	HydroSpeed™ equipped with 96HT/96-i wash heads
Wash program	**Parameters**	**Parameters**
96-well Microlon 600 flat-bottom plate (Greiner Bio One), or similar	Polystyrene plate with high binding surface properties	Polystyrene plate with high binding surface properties
Plate definition file:	[GRE96ft]	[GRE96ft]
Name:	**ELISA**	**ELISA_96**
Cycle 1:	# of cycles: 3	# of cycles: 3
Wash	- Mode: normal (one asp. point/well) - z-pos: bottom - Asp. time: 1 sec - Aspirate rate: 3 - Head speed: 10 mm/s	- Mode: normal (one asp. point/well) - z-pos: bottom - Asp. time: 1 sec - Aspirate rate: 3-5 - Head speed: 10 mm/s
	- z-pos.: overflow - disp. vol.: 450 µl/well - channel: 1 - disp. rate: 300 µl/sec	- z-pos.: overflow - disp. vol.: 450 µl/well - channel: 1 - disp. rate: 280 µl/s (96HT); resp. 225 µl/s (96-i wash head)
Cycle 2:	# of cycles: 1	# of cycles: 1
Aspirate	- Mode: crosswise (two asp. points/well) - z-pos: bottom - Asp. time: 2 sec - Aspirate rate: 3 - Head speed: 10 mm/s	- Mode: crosswise (two asp. points/well) - z-pos: bottom - Asp. time: 2 sec - Aspirate rate: 3-5 - Head speed: 10 mm/s

Typical ELISA wash program for the 384-well plate format using a HydroSpeed equipped with the 384HT wash head

HydroSpeed	
Wash program	**Parameters**
384-well clear, flat-bottom microplate (Greiner Bio One), or similar	Polystyrene plate with high binding surface properties
Plate definition file:	[GRE384ft]
Name:	**ELISA 384**
Cycle 1:	# of cycles: 3
Wash	- Mode: normal (one asp. point/well) - z-pos: bottom - Asp. time: 1 sec - Aspirate rate: 3-5 - Head speed:10 mm/s
	- z-pos.: overflow - disp. vol.: 350 µl/well - channel: 1 - disp. rate: 100 µl/sec
Cycle 2:	# of cycles: 1
Aspirate	- Mode: normal (one asp. point/well) - z-pos: bottom - Asp. time: 2 sec - Aspirate rate: 3-5 - Head speed:10 mm/s

Typical ELISA targets/assays and common providers

There is a wide range of different ready-to-go ELISA products on the market. One category focuses on diagnostic targets, like various virus-related proteins, another on more research-related targets, such as cytokines, interleukins, or cell death-related proteins. The links below present a small overview of available providers:

- **R&D Systems**
  www.rndsystems.com
- **Thermo Scientific – Pierce protein biology products**
  www.piercenet.com
- **Abcam**
  www.abcam.com
- **BioRad**
  www.bio-rad.com
- **BD Biosciences**
  www.bdbiosciences.com
- **DiaSorin**
  www.diasorin.com
- **Life Technologies**
  www.lifetechnologies.com
- **Millipore**
  www.millipore.com
- **EuroImmun**
  www.euroimmun.com

Support

Links

- Free article: The enzyme-linked immunosorbent assay (ELISA); Bull World Health Organ. 1976; 54(2): 129-139. www.ncbi.nlm.nih.gov/pmc/articles/PMC2366430/pdf/bullwho00453-0009.pdf
- Free article: Stephanie D. Gan and Kruti R. Patel; Enzyme Immunoassay and Enzyme-Linked Immunosorbent Assay; Journal of Investigative Dermatology (2013) 133, e12. doi:10.1038/jid.2013.287 www.nature.com/jid/journal/v133/n9/pdf/jid20132287a.pdf

Tecan application note:

- Application Note: Fast and efficient processing of ELISA assays using Tecan's HydroSpeed plate washer and Infinite® F50 absorbance reader; 396538 V1.0, 12-2010

Fast & efficient processing of ELISA assays

using Tecan's HydroSpeed™ plate washer and Infinite® F50 absorbance reader*

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC1	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Pos
B	NC2	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
C	NC3	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg
D	NC4	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg
E	PC1	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
F	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Neg	Neg
G	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
H	Pos	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Pos

Figure 1: Plate layout. NC1-4 = negative control; PC1 = positive control; Pos = positive patient samples; Neg = negative patient samples.

Introduction

This application note describes the outcome of a successful evaluation study of Tecan's new HydroSpeed plate washer and Infinite F50 absorbance reader using a HBs Ag assay. This assay is used as an example of a qualitative, one step 'sandwich' type enzyme immunoassay for the detection of hepatitis B viral surface antigen (HBs Ag) in human serum or plasma (1).

Hepatitis B is an infectious disease, caused by the hepatitis B virus (HBV), which infects the human liver causing an inflammation. Transmission of the virus occurs by exposure to infected blood or other infected body fluids, and about one third of the world's population is infected with the HBV. The HBs Ag assay typically allows to detect the HBs Ag in human serum or plasma, as this is the first marker to appear after infection, and can be detected two or three weeks before the first clinical and biological symptoms of the disease (1). Because hepatitis B represents a serious transfusion hazard, the detection of HBV is one of the most important routine clinical tests performed worldwide.

It is crucial to detect the HBs Ag at the time of blood donation to prevent it being transmitted during a transfusion.

Tecan's new HydroSpeed plate washer offers advanced features for a range of applications including vacuum filtration, magnetic bead separation and washing of ELISA- and cell-based assays in 96- & 384-well plate format. Reliable operation is provided by the HydroSpeed's automated Anti-Clogging™ function, which prevents needle blockage during idle time between plates, typically caused by crystallization of the wash buffer.

The Infinite F50 absorbance reader is Tecan's state-of-the-art, 8-channel absorbance microplate reader that provides not only accurate, reproducible and fast measurements, but sets new standard in compact and innovative design. Together with Magellan™, Tecan's easy-to-use control and data analysis software, the Infinite F50 is ideal for a variety of ELISA applications.

The HydroSpeed plate washer and the Infinite F50 absorbance reader are a perfect combination for ELISA-based assays, offering high throughput capacity and accurate data acquisition/analysis, fulfilling the major requirements for clinical and research laboratories.

* Caution: This note describes a combination of automation and reagent kit that has not been cleared or approved by regulatory authorities including the United States Food and Drug Administration. Consult your local regulatory authority prior to implementation of automation for any IVD application.

Materials and methods

Instruments

- HydroSpeed plate washer equipped with the 96-HT or 96-indexing wash head for ELISA and cell washing
- Infinite F50 absorbance reader

Microplate

- NUNC® MaxiSorp™ 96-well strip plate (provided with the kit)

Reagents

- HBs Ag ELISA kit

Assay procedures

The HBs Ag ELISA detects a small part of the surface antigen of the hepatitis B virus at low sample concentrations and uses an alkaline wash buffer and tetramethylbenzidine (TMB) for color detection. Please refer to the assay description for detailed information and an explanation of the principle of this ELISA assay (1).

The assay was performed according to the manufacturer's assay manual, using the kit's negative and positive controls in addition to 15 positive and 76 negative patient serum samples (figure 1). All washing steps were performed with the HydroSpeed plate washer equipped with the 96-HT wash head or the 96i wash head, and the readout was performed with the Infinite F50 absorbance reader. For detailed information on the washing program and the measurement settings, please refer to figure 2 and table 1.

Measurement settings and wash program

Measurement parameters	
Plate definition file	NUN96ft.pdfx
Wavelength	450 nm
Reference wavelength	620 nm

Table 1: Measurement parameters for Infinite F50 absorbance reader.

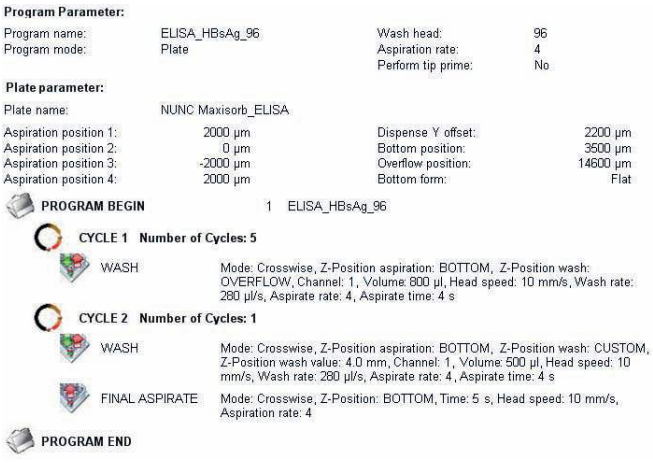


Figure 2: Wash program for the HydroSpeed plate washer equipped with the 96-HT wash head. For the 96i wash head the same program with a wash rate of 180 µl/s was used.

Results and discussion

Interpretation of assay results (according to the kit manual):

1. All negative control values (NC1 - NC4) must give an absorbance value ≤ 0.08 OD
2. Each positive control must give an absorbance value ≥ 1.0 OD
3. Cut-off value: NCmean + 0.05
4. Calculation of ratio: OD of sample / OD of cut-off
   Ratio > 1: sample is positive
   Ratio < 0.9: sample is negative
   Ratio > 0.9 and < 1: sample is negative but has to be retested.

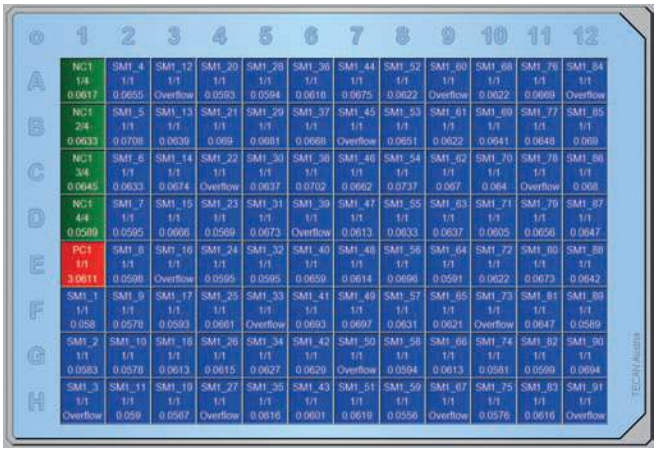


Figure 3: Magellan - raw data.

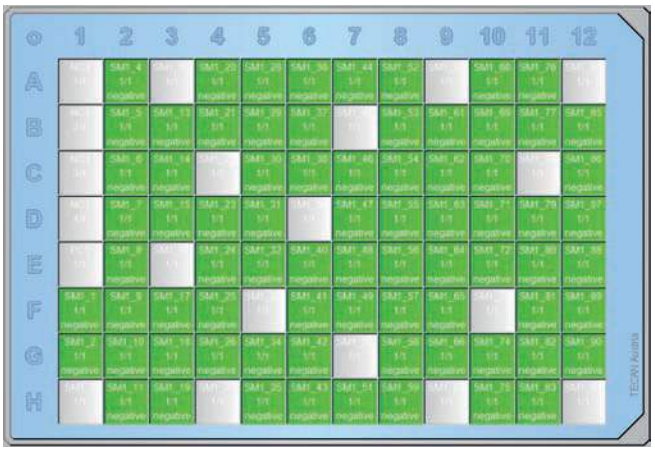


Figure 4: Magellan - cut-off results.

Figures 3 and 4 show the raw data and final cut-off results obtained from Magellan software using the HydroSpeed plate washer with the 96-HT wash head. All negative controls show absorbance values below 0.08 OD, and all positive controls show absorbance values above 1.0 OD. All positive and negative patient samples were correctly detected, and no false positive or false negative results occurred. None of the samples needed retesting. Results for the 96i wash head are not shown, but were comparable to the data listed above.

Note: All positive patient samples were used without pre-dilution of the probe; therefore raw data values are displayed as 'Overflow' (OD > 4). As a consequence these wells were not marked red, which is the typical indication of positive samples.

Conclusion

The results presented in this application note clearly demonstrate that the HydroSpeed plate washer and the Infinite F50 absorbance reader are well suited for fast & efficient processing of ELISAs, such as a HBs Ag assay.
The HydroSpeed plate washer provides outstanding washing performance, enabling critical samples to be handled, such as the undiluted serum probes used in this study. The 96-channel wash head provides the HydroSpeed plate washer with the capability to perform high throughput ELISA washing.
This matches the concept of the Infinite F50 absorbance reader, which is equipped with an 8-channel absorbance optic that enables ultra-fast absorbance measurements of less than 20 seconds for a 96-well plate.

Literature

(1) www.biorad.com

List of Abbreviations

HBs Ag	surface antigen of Hepatitis B virus
NC	negative control
Neg	negative patient samples
PC	positive control
Pos	positive patient samples
TMB	tetramethylbenzidine
OD	optical density
HT	high throughput

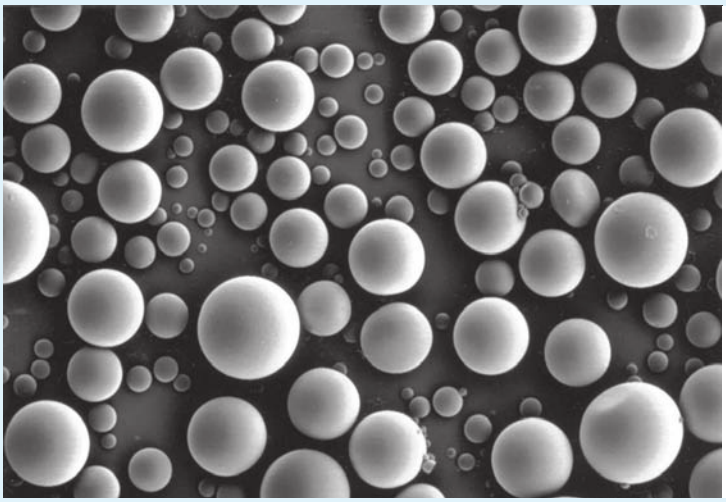
Acknowledgements

We would like to express our thanks to Mag. Dr. Tobias Kiesslich and Josef Lang (Department of Internal Medicine I, Paracelsus Medical University and SALK, Salzburg, Austria) for their collaboration and for performing these experiments.

Groedig, Austria, December 2010

396538 V1.0, 12-2010

Vacuum filtration



Array of beads

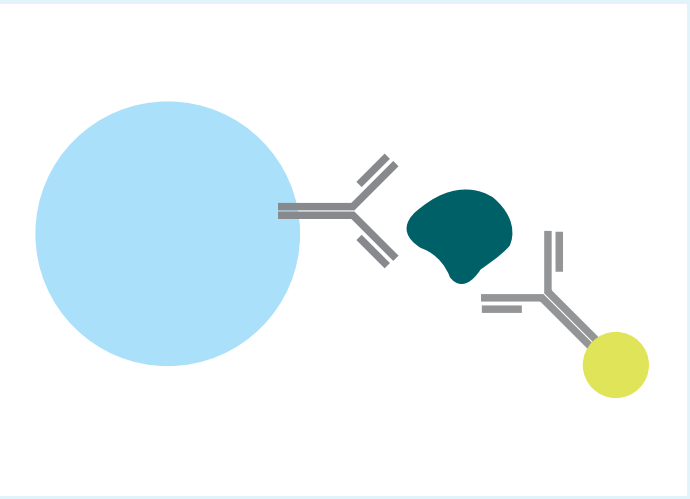


Figure 1: Schematic illustration of a bead-based ELISA.

Principle

ELISAs are continuously evolving to meet increasing user demands, such as the need for multiplexed assays which reduce the cost and time per assay by producing several results per assay well.

One enhancement is the use of microspheres (beads) as a solid support for the assay. Beads have the advantage of providing a very large reaction surface area, rather than just the surface of each well (figure 1).

This increase in surface area is enhanced even further by an even distribution of beads throughout the well, resulting in a higher reaction speed and a significantly lower background signal, offering a higher level of sensitivity compared to traditional ELISAs.

Most bead-based multiplex assays are provided as panels, for example the xMAP® technology from Luminex, which theoretically allows up to 100 multiplexed assays per sample. This technique takes advantage of color-coded bead sets, which are created by labeling the beads using different ratios of two fluorescent dyes. Each of these beads is characterized by its individual color - and each color corresponds to a distinct analyte.

To set up the assay, each colored bead is combined with a specific set of capture reagents – such as antibodies – that will interact with an analyte of interest, as well as any reagents specific to a particular bioassay, including antigens, antibodies, oligonucleotides, enzyme substrates or receptors.

During incubation with the sample, the analytes bind to their corresponding capture reagents. Afterwards, a vacuum filtration step using a filter-bottom plate is performed to remove unbound fractions of the sample.

An incubation step with a set of detection antibodies (typically labelled with fluorescent reporters) is then performed. These detection antibodies bind to their matching analytes with high specificity, then another vacuum filtration step is performed to remove any unbound detection antibodies.

Detection is carried out using a flow cytometer; one bead after another is sent through a capillary element and is interrogated using two different lasers. The first laser is used for the identification of the bead via its individual color code.

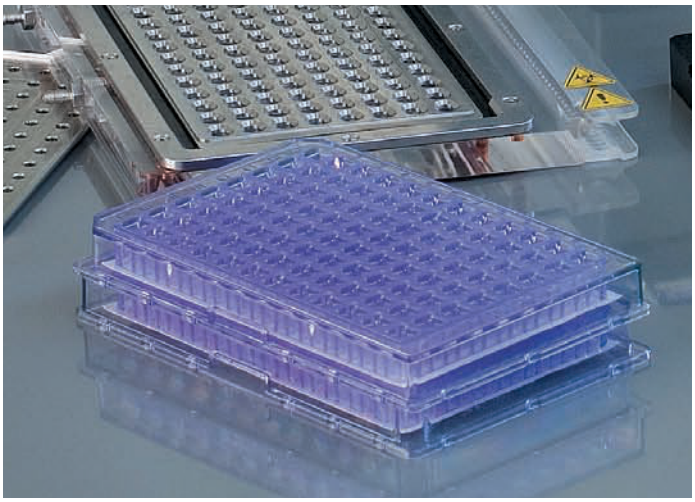


Figure 2: Typical filter-bottom plates used for vacuum filtration.

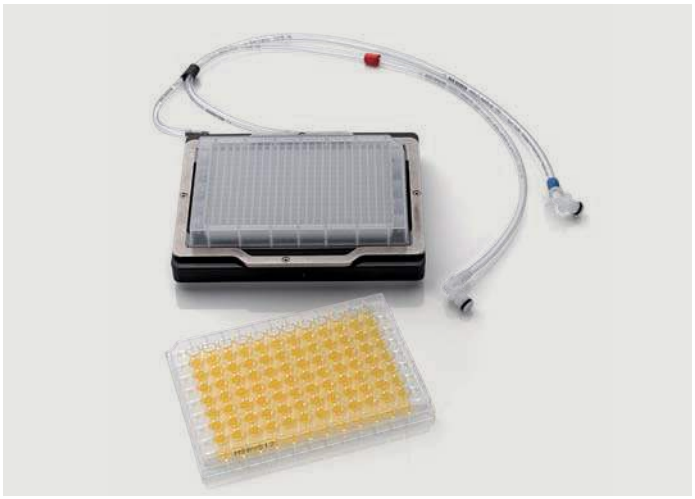


Figure 3: Filtration plate carrier which is part of the vacuum filtration option for the HydroSpeed plate washer.

The second laser interacts with the fluorescent label on the detection antibody to produce both qualitative and quantitative fluorescent measurements.

In general, there are two types of beads available; i) non-magnetic beads made from latex, silicone or polystyrene, and ii) magnetic beads. Protocols for non-magnetic bead-based assays use vacuum filtration with filter-bottom microplates (figure 2), whereas magnetic beads are typically washed in a magnetic field to remove unbound substances (see next chapter for magnetic bead washing).

Critical parameters

Manual washing of non-magnetic beads is very tedious and time consuming, and is prone to errors – such as leaking of individual wells during incubation, clogging of wells during the filtration of viscous serum samples and bead loss into the pores of the filter membrane due to high vacuum settings – which may lead to inconsistent results. Typically a generic vacuum setting has to be used for the entire filtration protocol, regardless of the changing sample viscosity during the filtration protocol, further reducing assay performance.

The use of a microplate washer equipped with a vacuum filtration option only partially overcomes these limitations, as typically the vacuum level can be set only once for the entire protocol.

Tecan solution

However, Tecan's HydroSpeed and HydroFlex washers overcome this limitation by allowing the adjustment of the vacuum level individually for each filtration step within a protocol, in ranges from -50 to -300 mbar and from to -50 to -150 mbar, respectively. This helps to optimize bead recovery and filtration efficiency, by only using a strong vacuum setting for the initial filtration step, when the typical viscosity of the sample is high. Following addition of wash buffer after the initial filtration, once the viscosity of the sample has dropped, you can use a more gentle vacuum level for subsequent filtrations to avoid loss of beads into the pores of the filter membrane.

The HydroFlex washer is also available in a high-level vacuum configuration, offering an extended vacuum range from -150 to -850 mbar, making it the solution of choice for high-vacuum applications, such as automated DNA purification to remove salts and oligos after a PCR step.

Washer comparison for vacuum filtration

Washer:	HydroFlex™	HydroSpeed™
96-well filter-bottom plates	x	x
384-well filter-bottom plates		x
Vacuum control: -50 to -150 mbar for filter washing of non-magnetic beads	x	
Vacuum control: -50 to -300 mbar for filter washing of non-magnetic beads		x
High level vacuum control: -150 to -850 mbar for DNA purification after PCR	x	
Automation-friendly design (without manually operated clamp mechanism to fix plate onto the filtration plate carrier)	x	x
Capability to define the vacuum level individually for each filtration step	x	x
Automated bleed valve for vacuum release after the filtration step – allows fast unloading of plates	x	x

Typical wash program for high-level vacuum filtration with HydroFlex for DNA purification after PCR in a 96-well plate format

Wash program	Parameters
96-well MultiScreen PCR µ96 (Merck Millipore)	Filter membrane with 0.45 µm pore size
Plate definition file:	[Mill96PCR]
Cycle 1:	# of cycles: 1
Vacuum Filtration	- time: 3-7 minutes - vacuum: -800 mBar

Recommendation

After the filtration protocol has been completed, blot remaining droplets from the bottom of the filter plate using absorbant material.

For proper build-up of the vacuum, all wells of the filtration plate should be filled with liquid.

If performing vacuum filtration with the HydroFlex on low sample numbers, meaning that only a part of the filtration plate is used; all unused wells should be sealed with Parafilm.

Typical wash program for vacuum filtration of a multiplexed, bead-based ELISA in a 96-well plate format

	HydroFlex™ with low-level vacuum filtration option	HydroSpeed™ with vacuum filtration option and 96i / 96HT wash head
Wash program	**Parameters**	**Parameters**
96-well MultiScreen® filtration plate (Merck Millipore)	Filter membrane with 1.2 µm pore size	Filter membrane with 1.2 µm pore size
Plate definition file:	[Mill96ft]	[VAC_Mill96ft]
Name:	**Pre-Wet**	**Pre-Wet**
Cycle 1:	# of cycles: 1	# of cycles: 1
Dispense	- z-pos.: overflow - disp. volume: 100 µl/well - channel: 1 - disp. rate: 300 µl/sec	- z-pos.: overflow - disp. volume: 100 µl/well - channel: 1 - disp. rate: 180 µl/sec (96i wash head) resp. 280 µl/sec (96HT wash head)
Soak	- time: 20 sec	- time: 20 sec
Vacuum Filtration	- time: 20 sec - vacuum: -70 mBar - clean: activated	- time: 20 sec - vacuum: -70 mBar - clean: activated
Name:	**Example_Vac**	**Example_Vac**
Cycle 1:	# of cycles: 1	# of cycles: 1
Vacuum Filtration	- time: 40 sec - vacuum: -150 mBar - clean: not active	- time: 40 sec - vacuum: -150 mBar - clean: not active
Dispense	- z-pos.: overflow - disp. volume: 100 µl/well - channel: 1 - disp. rate: 300 µl/sec	- z-pos.: overflow - disp. volume: 150 µl/well - channel: 1 - disp. rate: 180 µl/sec (96i wash head) resp. 280 µl/sec (96HT wash head)
Cycle 2:	# of cycles: 1	# of cycles: 1
Vacuum Filtration	- time: 30 sec - vacuum: -70 mBar - clean: not active	- time: 20 sec - vacuum: -70 mBar - clean: not active
Dispense	- z-pos.: overflow - disp. volume: 100 µl/well - channel: 1 - disp. rate: 300 µl/sec	- z-pos.: overflow - disp. volume: 150 µl/well - channel: 1 - disp. rate: 180 µl/sec (96i wash head) resp. 280 µl/sec (96HT wash head)
Cycle 3:	# of cycles: 1	# of cycles: 1
Vacuum Filtration	- time: 30 sec - vacuum: -70 mBar - clean: active	- time: 20 sec - vacuum: -70 mBar - clean: active

Typical beads assays and common providers

To date there are several different technologies and providers for bead-based ELISAs available. Here is a selection of the most common technologies.

- **Life Technologies**
  www.lifetechnologies.com
  - Dynabeads® magnetic separation technology

- **Luminex**
  www.luminexcorp.com
  - xMAP technology

- **Millipore**
  www.luminexcorp.com
  - MILLIPLEX® MAP cell signaling technology based on Luminex
  - xMAP technology

- **Bio-Rad**
  www.bio-rad.com
  - Bio-Plex Pro™ technology based on Luminex xMAP technology

- **Thermo Scientific – Pierce protein biology products**
  www.piercenet.com

Support

Links

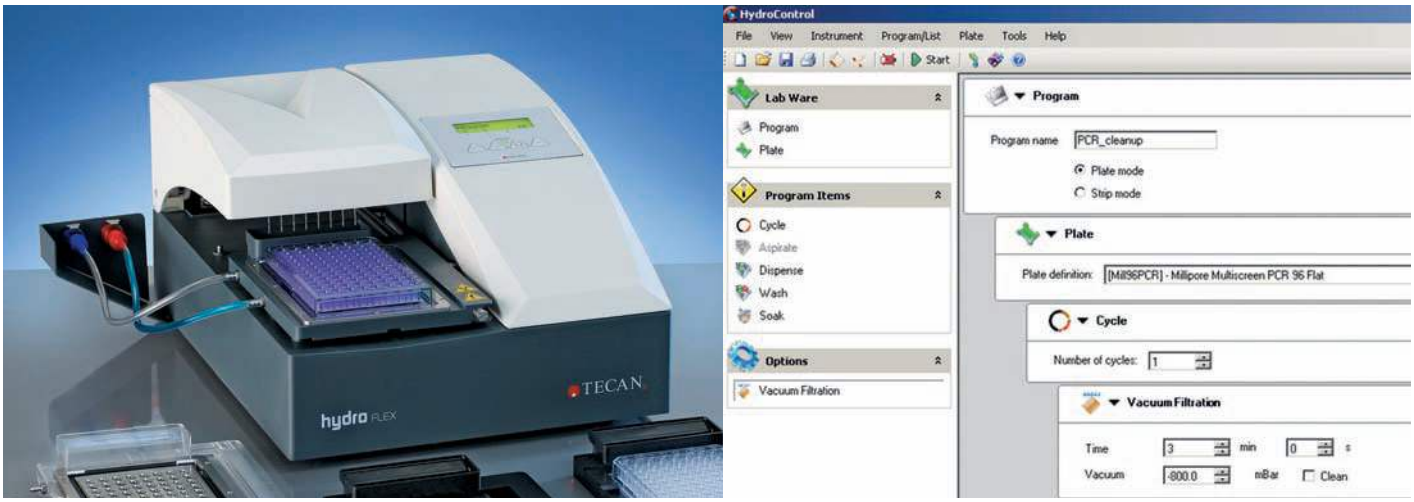
- Free article: Sherry A. Dunbar, Applications of Luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection, Clinica Chimica Acta, Volume 363, Issues 1-2, January 2006, Pages 71-82
  dnatech.genomecenter.ucdavis.edu/documents/applications_of_luminex.pdf
- Free article: Eleonora Codorean et al, Correlation of xMAP and ELISA cytokine profiles; development and validation for immunotoxicological studies in vitro, Romanian archives of microbiology and immunology, Volume 69 – No. 1
  www.roami.ro/files/RAMI_1_2010.pdf#page=5

Tecan technical note:

- Technical Note: automated PCR product purification for increased productivity using the HydroFlex platform with the vacuum filtration option; 395 083 V1.0, 05-2007

Automated PCR-product purification for increased productivity

Using the HydroFlex™ platform with the high level vacuum filtration option



Introduction

Purification of biomolecules requires a series of steps involving the removal of contaminants, desalting, buffer exchange, and sample concentration.
Automated vacuum filtration technology for purification has a number of advantages in simultaneous processing of multiple samples, speed and consistency, as well as retaining the biological activity of the biomolecule of interest.
For many downstream applications, such as sequencing or hybridization, PCR-amplified DNA has to be purified to remove primers or other components from the reaction mix.
To increase productivity of vacuum filtration protocols, the HydroFlex automates key process steps including self-sealing of filtration plates, online vacuum control, fast dispensing of wash buffers, and many more.
Additionally, the HydroFlex offers a tuneable vacuum range from -150 mbar to -850 mbar relative pressure, making it the solution of choice for high-vacuum applications such as automated PCR-cleanup, as well as for low-vacuum applications such as processing of bead-based assays.
Furthermore, the HydroFlex provides online control of the vacuum level for fast initiation and cessation of filtration and makes manual vacuum pre-calibration a thing of the past.

This technical note describes the purification of PCR products via automated vacuum filtration using the modular Tecan HydroFlex platform equipped with the vacuum filtration option.

Materials and methods

Equipment
- HydroFlex platform equipped with an 8-way manifold and high level vacuum filtration option (Tecan Austria, Austria)
- Microplate shaker
- Infinite® M200 (Tecan Austria)

Materials
- 1000 bp PCR product (University of Salzburg, Austria)
- 96 well filtration plate Montage® PCRµ96 LSKM PCR10 (Millipore, MA)
- Nuclease free water (Carl Roth GmbH, Karlsruhe)
- SYBR® Green (10000 x stock solution, FMC Bio Products, ME, USA)

Method
To quantify the DNA-recovery after vacuum filtration via a fluorescence intensity measurement, 4.2 ml of amplified PCR products and 16.8 ml nuclease free water were mixed with 0.42 µl SYBR Green.

For the purification of the amplified DNA after the PCR-step, 100 µl PCR-product mix per well was pipetted into the 96-well filtration plate.
The filtration plate was placed on the HydroFlex vacuum filtration station and then an automated vacuum filtration protocol as mentioned below was started.
A vacuum pressure of -800 mbar was applied to the filter-bottom plate for 3 min to collect the amplified DNA on top of the filtration membrane and to remove unwanted components together with the filtrate.
If required from the assay, the HydroFlex can automatically perform protocols consisting of repeated vacuum filtration and dispense steps to "wash" the amplified DNA more thoroughly.
For DNA recovery the plate was removed from the HydroFlex vacuum filtration station and 50 µl of Nuclease-free TE-buffer or water were added to each well covering the surface of the filtration membrane. Then the filtration plate was agitated for 10 min on an MTP-shaker at 1100 rpm to re-suspend the purified DNA.
Supernatants of the re-suspended and pre-labelled DNA were transferred into a 96-well flat bottom plate to measure the fluorescence intensity (λ_{ex} 485 nm / λ_{em} 535 nm) using a Tecan Infinite M200 reader.
Recovery rates were calculated in relation to the SYBR Green-based fluorescence of the PCR products which were measured before and after the vacuum filtration.

Results and Discussion

A total of 96 amplified DNA-samples after the PCR-step were purified using the HydroFlex automated vacuum filtration station and a MultiScreen PCR96 filtration plate.
The automated vacuum filtration protocol using the HydroFlex showed good results and high recovery rates above 80% were achieved for the purification of amplified DNA of 1000 bp in length (figure 1 and 2).
The purified DNA obtained after the vacuum filtration with the Tecan HydroFlex platform is ready for use for further applications, such as hybridization or sequencing.

Vacuum filtration with Tecan HydroFlex: -800 mbar for 3 min			
			Standard deviation
Recovery rate	Run 1	81.63 %	4.81
	Run 2	80.29 %	8.36
Raw data [RFU]	Run 1	33871.8	1965.5
	Run 2	33315.7	3450.4

Figure 1: Recovery results after vacuum filtration of DNA (-800 mbar, 3 min)

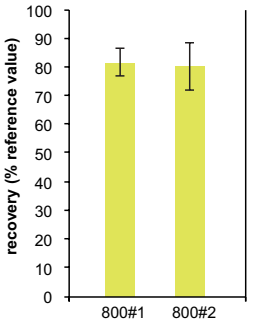


Figure 2: Recovery rate of SYBR® Green-labelled DNA after purification via vacuum filtration (800 mbar, 3 min) using Tecan's HydroFlex platform

Conclusion

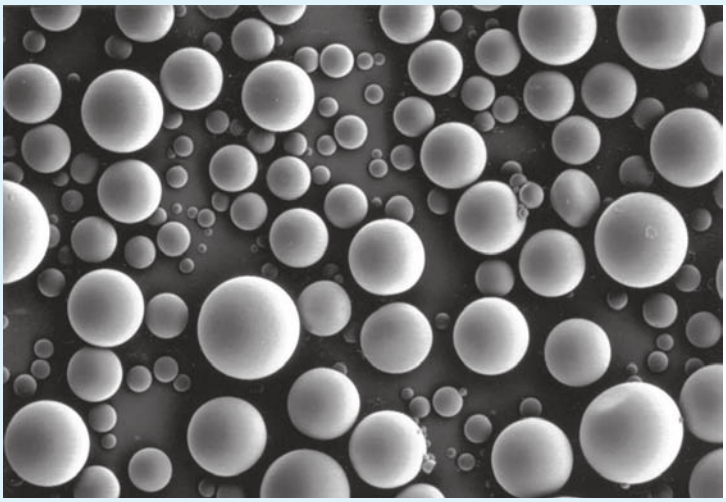
The HydroFlex platform equipped with the vacuum filtration option combines good recovery rates with high productivity via automation of key process steps for the purification of amplified DNA after the PCR-step.
With the HydroFlex platform, tedious protocol steps such as the pre-calibration of the vacuum level, the manual initiation and cessation of the filtration steps, and the manual dispensing steps associated with the classical way of vacuum filtration, are a thing of the past.
Tecan's HydroFlex Washer platform is a compact 3-in-1 solution designed to automate a range of applications including vacuum filtration, magnetic bead separation and the washing of microplates.
The compact HydroFlex vacuum filtration station can be used as a stand-alone system or can be easily integrated on a Tecan workstation for fast processing of larger amounts of samples.

Acknowledgements

We would like to thank Mag. Tobias Kiesslich, Mag. Juergen Berlanda (Research Group Prof. B. Krammer), Department of Molecular Biology, University of Salzburg
www.uni-salzburg.at/pdt
May, 2007

395 083 V1.0, 05-2007

Magnetic bead washing



Array of beads

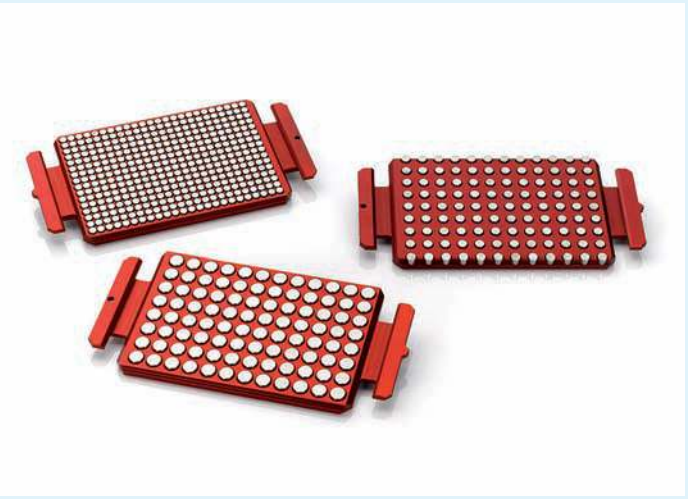


Figure 1: A selection of magnetic carriers for optimized magnetic bead washing in 96- and 384-well plates on the HydroSpeed plate washer: MBS 384 carrier (top left), Smart-2 MBS 96 (middle) and MBS 96 (bottom).

Principle

The latest generation of multiplexed assays is based on polystyrene beads, which are partly coated with iron oxide to give them magnetic properties. Luminex magnetic beads are a typical example of this.

All magnetic bead-based ELISA protocols require the beads to be washed in a magnetic field to remove the fractions of sample that have not bound to specific capture antibodies immobilized on the surface of the beads. During the wash cycles a magnetic field is used to settle the beads and fix them prior to aspiration, to avoid bead loss.

Critical parameters

Performing the washing stages manually is tedious and understandably very prone to errors. It entails loading the plate onto a magnetic stand, waiting for the beads to settle, and then removing the supernatant from each well using a multichannel pipette. At this stage, an operator has little control over the position of the pipette tips inside the wells, and can easily pick up beads accidentally while aspirating the supernatant, leading to inconsistent results.

The operator then has to unload the plate from the magnetic stand and fill each well with wash buffer, again using a multichannel pipette. Many ELISA protocols using magnetic beads include several wash cycles, and so tackling the washing stages manually creates a significant bottleneck.

Tecan solution

The HydroFlex and HydroSpeed plate washers alleviate these problems by automating magnetic bead washing, and have the added advantage of being compatible with Tecan robotic platforms, which can automate the entire assay workflow to boost productivity and improve the consistency of results.

Tecan offers dedicated magnetic plate carriers equipped with an array of powerful rare-earth magnets for both the HydroFlex and the HydroSpeed plate washers, covering a range of applications and bead sizes.

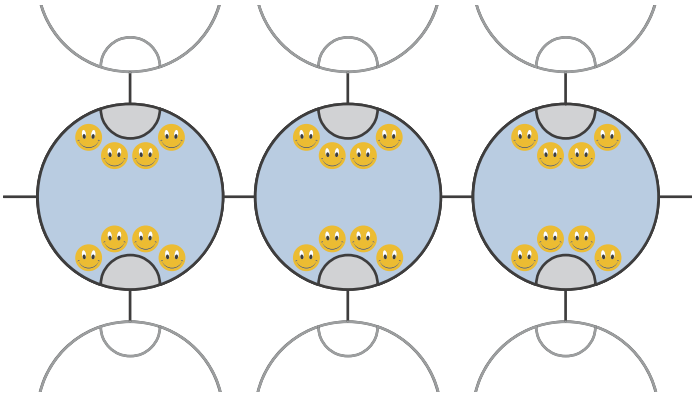


Figure 2: Schematic principle of the patent-pending Smart-2 MBS 96 magnetic carrier for the HydroFlex and HydroSpeed plate washers. Magnetic beads are moved to the sides of the flat-bottom wells using two powerful rare earth magnets, providing excellent bead recovery.

The Smart-2 MBS carrier is equipped with two powerful magnets per well that effectively settle the beads outside of the aspiration zone, helping to protect them during washing in a 96-well plate format (figure 2). This patent-pending design helps to optimize results by maintaining high bead recovery rates and keeping residual volumes low.

HydroFlex magnetic bead washing in 96-well plate format	
Smart-2 MBS magnetic carrier with two magnets per well	For medium sized magnetic beads such as Luminex and M280 Dynabeads
MBS 96 magnetic carrier with one large magnet per well	For small magnetic beads such as µOne Dynabeads

HydroSpeed magnetic bead washing in 96- and 384-well plate formats	
Smart-2 MBS magnetic carrier with two magnets per well	For medium sized magnetic beads such as Luminex and Dynabeads M-280
MBS 96 magnetic carrier with one large magnet per well	For small magnetic beads such as µ-One Dynabeads
MBS 384 magnetic carrier with one magnet per well	For a range of magnetic beads such as Luminex and Dynabeads

The standard MBS magnetic carriers listed above incorporate one extra-strong rare earth magnet per well to settle even small magnetic beads such as µ-One Dynabeads with a typical bead diameter of 1 µm effectively, and are available for 96-well and 384-well plates (figure 1).

As well as the magnetic bead carriers, the HydroFlex and HydroSpeed plate washers are ideal for magnetic bead washing because they provide full control over critical parameters. These include bead settling times, vacuum power, wash head speed, and fine-adjustment of the wash head position, allowing gentle aspiration for optimized bead recovery rates. They also offer higher dispense speed settings and shaking capabilities to help with bead mixing.

Washer comparison for magnetic bead washing

Washer:	HydroFlex™	HydroSpeed™
96-well plates	x	x
384-well plates		x
MBS 96 magnetic carrier	x	x
MBS 384 magnetic carrier		x
Smart-2 MBS 96 magnetic carrier	x	x
Aspiration fine tuning	x	x
Dispensing fine tuning	x	x
Microplate shaking	x	x
Aspiration height control	x	x
Control of aspiration power	x	x

Typical wash program for multiplexed assays based on Luminex beads in 96-well plate format

	HydroFlex™ with Smart-2 MBS magnetic plate carrier	HydroSpeed™ with Smart-2 MBS carrier and 96HT wash head
Wash program	**Parameters**	**Parameters**
96-well µ-clear plate (Greiner Bio One, article code 655096)	Low bottom thickness of the wells of 0.3 mm only	Low bottom thickness of the wells of 0.3 mm only
Plate definition file:	[GRE96fb_magbeads]	[MAG_GRE96ft]
Name:	**MAG**	**MAG_96**
Cycle 1:	# of cycles: 1	# of cycles: 1
Soak (bead settling)	- time: 90 sec	- time: 90 sec
Aspirate	- Mode: normal (one asp. point / well) - z-pos: custom 6 mm - Asp. time: 1 sec - Aspirate rate: 1 - Head speed: 8 mm/s	- Mode: normal (one asp. point / well) - z-pos: custom 6 mm - Asp. time: 1 sec - Aspirate rate: 1 - Head speed: 2 mm/s
Dispense	- z-pos.: overflow - disp. volume: 200 µl/well - channel: 1 - disp. rate: 300 µl/sec	- z-pos.: overflow - disp. volume: 200 µl/well - channel: 1 - disp. rate: 350 µl/sec
Cycle 2:	# of cycles: 1	# of cycles: 1
Soak	- time: 60 sec	- time: 60 sec
Aspirate	- Mode: normal (one asp. point / well) - z-pos: custom 6 mm - Asp. time: 1 sec - Aspirate rate: 1 - Head speed: 8 mm/s	- Mode: normal (one asp. point / well) - z-pos: custom 6 mm - Asp. time: 1 sec - Aspirate rate: 1 - Head speed: 2 mm/s
Dispense	- z-pos.: overflow - disp. volume: 200 µl/well - channel: 1 - disp. rate: 300 µl/sec	- z-pos.: overflow - disp. volume: 200 µl/well - channel: 1 - disp. rate: 350 µl/sec
Cycle 3:	# of cycles: 1	# of cycles: 1
Soak	- time: 60 sec	- time: 60 sec
Aspirate	- Mode: normal (one asp. point / well) - z-pos: cust. 5.5 mm - Asp. time: 1 sec - Aspirate rate: 1 - Head speed: 5 mm/s	- Mode: normal (one asp. point / well) - z-pos: cust. 5.5 mm - Asp. time: 1 sec - Aspirate rate: 1 - Head speed: 1 mm/s

Typical magnetic beads assays and common providers

To date, there are several different technologies and providers for magnetic beadbased ELISAs available. Here is a selection of the most common technologies.

- **Life Technologies**
  www.lifetechnologies.com
  - Dynabeads magnetic separation technology

- **Luminex**
  www.luminexcorp.com
  - xMAP technology, MagPlex Microspheres

- **Millipore**
  www.luminexcorp.com
  - Milliplex MAP Cell Signaling technology based on Luminex xMAP technology

- **Bioclone Inc**
  www.bioclone.us
  - BcMag™ Magnetic Beads

- **Bio-Rad**
  www.bio-rad.com
  - Bio-Plex Pro technology based on Luminex xMAP technology

- **Thermo Scientific – Pierce protein biology products**
  www.piercenet.com

Support

Links

- Free article: ZM Saiyed et al, Application of magnetic techniques in the field of drug discovery and biomedicine, BioMagnetic Research and Technology 2003, 1:2 doi:10.1186/1477-044X-1-2 www.biomagres.com/content/pdf/1477-044X-1-2.pdf
- Free article : Q A Pankhurst et al, Applications of magnetic nanoparticles in biomedicine, J. Phys. D: Appl. Phys. 36 (2003) R167-R181 www.physics.gla.ac.uk/~dtngo/Article/JPD_36_2003_R167.pdf
- Free article: Sherry A. Dunbar, Applications of Luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection, Clinica Chimica Acta, Volume 363, Issues 1-2, January 2006, Pages 71-82 dnatech.genomecenter.ucdavis.edu/documents/applications_of_luminex.pdf
- Free article: Eleonora Codorean et al, Correlation of xMAP and ELISA cytokine profiles; development and validation for immunotoxicological studies in vitro, Romanian archives of microbiology and immunology, Volume 69 - No. 1 www.roami.ro/files/RAMI_1_2010.pdf#page=5

Tecan application notes:

- Application note: Fast magnetic bead purification of interacting cellular proteins – implementation on Tecan's HydroFlex washer; 395 570 V1.0, 08-2008
- Application note: Efficient and gentle processing of magnetic Dynabeads using Tecan's HydroFlex microplate washer; 396 036 V1.0, 07-2009
- Application note: Efficient washing of magnetic beads – Using Tecan's HydroSpeed plate washer for EMD Millipore's MILLIPLEX MAP human cytokine/chemokine magnetic bead panel; 397403 V1.0, 12-2013

Fast Magnetic Bead Purification of Interacting Cellular Proteins

Implementation on Tecan's HydroFlex™ Washer

Introduction

Biomedical research, and in particular cancer research, is progressing from focusing on small numbers of molecules or cellular events to global functional analysis. Therefore, methods that allow one to look at a broader angle at cellular processes, such as mRNA expression levels or protein interaction patterns, are needed more and more often to study fundamental processes.

Coaffinity purification of two proteins from a complex mixture is one of the standard methods for the detection of protein-protein interactions.

To circumvent the need for specific antibodies in affinity purification and subsequent detection, proteins can be expressed in fusion with a "tag", i.e. an extension that has a high-affinity binding site for a generic antibody. When two differently tagged proteins are used, the first tag can be used for the specific purification of the complex, and the second tag for the detection of the co-purifying protein.

In one variation of this protocol, the tag for detection is not an antibody tag, but an enzyme that can be detected via its catalytic activity, such as luciferase [1] highly amplifying the read out signal. This avoids the need of gels and blots for detection of the tag, and allows microplate based miniaturization and automation of the protocol (Figure 1).

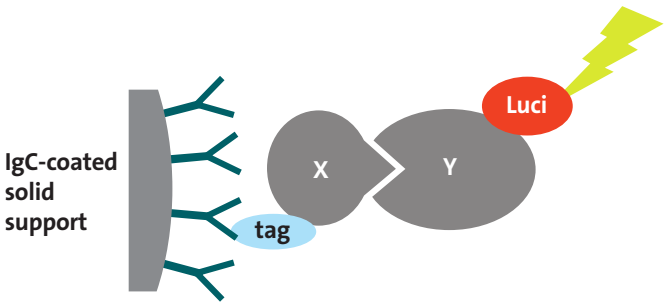


Figure 1: Principle of luciferase-based co-precipitation assays. The interaction of two proteins, X and Y, is tested. Protein X is purified over a solid support via an affinity tag, such as coated microplates or magnetic beads. The presence of protein Y is measured via the luciferase tag. Co-purification of protein Y with protein X is an indication for a direct or indirect association of the two proteins.

In this application note we describe the use of Tecan's HydroFlex™ washer equipped with a magnetic bead plate carrier for fast purification of a large number of samples using magnetic beads. For the detection of the luminescence signal Tecan's Infinite® F200 multimode reader was used. In the protocol optimized in our lab at the DKFZ in Heidelberg (Germany) the affinity tag is Staphylococcus aureus protein A, which can be purified on immunoglobulins as the affinity matrix.
The use of magnetic beads for immobilization of immunoglobulins is advantageous for miniaturization and automation of the assay.

The work flow of the experiment is depicted in Figure 2. After transient expression of the protein pair in tissue culture cells, a lysate is prepared and allowed to bind to immunoglobulin-coated magnetic beads. Non-binding proteins are removed from the beads in a washing step. Luciferase activity is determined in the starting material, as well as in the purified beads. The fraction of bound luciferase activity in the positive vs. the negative control is taken as readout for binding.

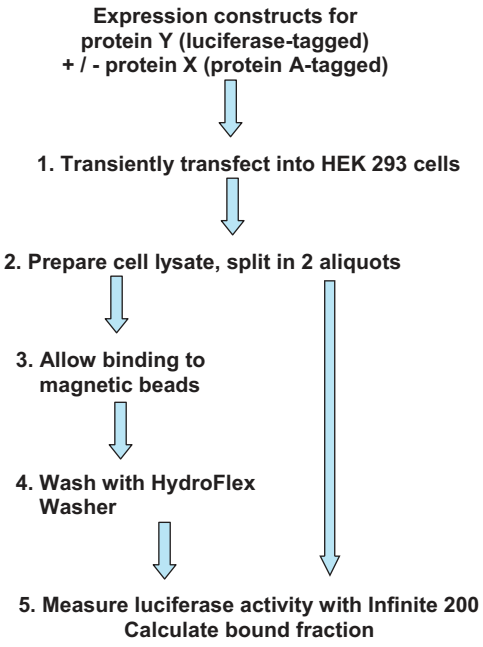


Figure 2: Workflow of a typical experiment. Negative controls can be included in step 1 (omission of the protein A–tagged protein), or in step 3 (use of non-immobilized immunoglobulin as a competing reagent, or use of non-specific beads). Steps 3 and 4 are omitted for part of the sample, which allows measuring the amount of luciferase in the input sample. Dividing bound activity by input activity corrects for input variations between samples.

An important step in such an automated protocol is the washing procedure used for purification of the protein complexes. The extensive options in the HydroFlex protocol allow finetuning of the washing procedure. The relative speed of the washing steps ensures a minimal loss of protein complexes during the wash, while maintaining a high enrichment factor, scan be measured via the activity of the luciferase tag.

Materials and methods

Instruments

- Tecan HydroFlex Washer equipped with 16 channel wash head, 4 inlet-channels for wash buffers and the Option smart-MBS carrier for washing of magnetic beads
- Tecan Infinite F200 multimode microplate reader

Microplates / beads

- 96-well microplate, flat bottom, white, LumiNunc™ (Nunc, Germany)
- Dynabeads® M-280 sheep anti rabbit

Assay Procedure

HEK293 cells were cotransfected with vectors directing the expression of Proteins fused to a Protein A-tag as well as a luciferasetag (Renilla luciferase). Cell extracts of transiently transfected HEK293 cells [1] were allowed to bind to magnetic beads coated with goat anti sheep IgG (Dynal) for two hours before washing with icecold PBS using the Tecan HydroFlex. Washed beads were resuspended in 60 µl PBS 1 mM DTT. Luciferase activity associated with the washed beads was determined using a Tecan Infinite F200 multimode reader (Attenuation: none, Settle time: 0 sec, Integration time: 1000 ms).

Instrument Settings

Wash Programm	1	2
Z-position	overflow	overflow
Volume	300 µl	300 µl
Dispense rate	500 µl / s	500 µl / s
Soak time	10 s	60 s
Aspirate		
Mode	Normal	Normal
Z-position custom	2500 µm	8100 µm
Time	1 s	1 s
Head speed	10 mm / s	3 mm / s
Aspiration rate	2	1

Table 1: Wash programs tested for magnetic bead purification using the HydroFlex washer.

Results

While wash program 1 yielded an excellent enrichment as well as a low background signal there was a loss of bead material from the wells that was apparent by visual inspection. To avoid loss of beads, the following changes were introduced leading to wash program 2:

- After addition of the wash buffer, a soak time of 1 minute was added to allow the beads to re-attach to the magnets.
- The aspiration rate was lowered to 1
- The Z-position was elevated such that the liquid was not removed completely, leaving ~20 µl in each well.

To test how many cycles were needed for near complete removal of non-associated luciferase activity, we ran the identical samples with 1, 2, 3, 4 and 5 washing cycles. The results are shown in Figure 3. Without the need to re-suspend the beads from the well bottom during dispensing of the wash buffer, three wash cycles were sufficient to achieve an over 300 fold enrichment of bound material.

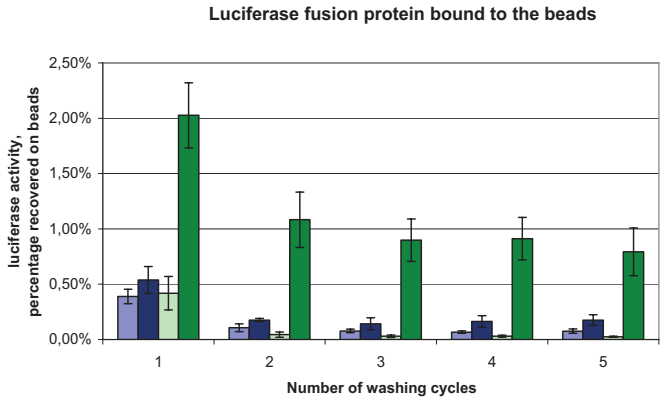


Figure 3: Testing the number of washing cycles. For testing we selected a weak interaction (CENPT-Renilla binding to RBBP7-protein A) ■ as a well as a strong interaction (SAP30-Renilla binding to SAP30-protein A) ■ Negative controls ■, ■ were luciferase-fusion proteins expressed in the absence of the protein A-tagged binding partner.

Typical binding stoichimetries of well characterized protein-protein interactions in these experiments range from 0.05% to 3% of Renilla-tagged protein recovered in a complex with the protein A-tagged protein. The very sensitive bioluminescence measurement allows the detection of weak interacting proteins and instable protein complexes.

Conclusion

The flexible programming of the washing steps possible with the HydroFlex instrument allowed a straightforward test of optimal washing conditions.

Washing efficiency is close to the theoretical optimal, despite the fact that most of the beads remain attached to the well bottom during the washes. The low number of washing cycles is crucial for the purification of instable protein complexes from cells. Purification can be done with the HydroFlex Washer in combination with the Infinite F200 multimode reader in a very short time. In this way it is possible to process up to ten plates (800 protein interaction assays) in less than an hour.

Acknowledgement

We express our acknowledgements to Dr. Manfred Koegl, German Cancer Research Center, Heidelberg (Germany) for the assay optimization, analysis of the data and for writing this note.

Literature

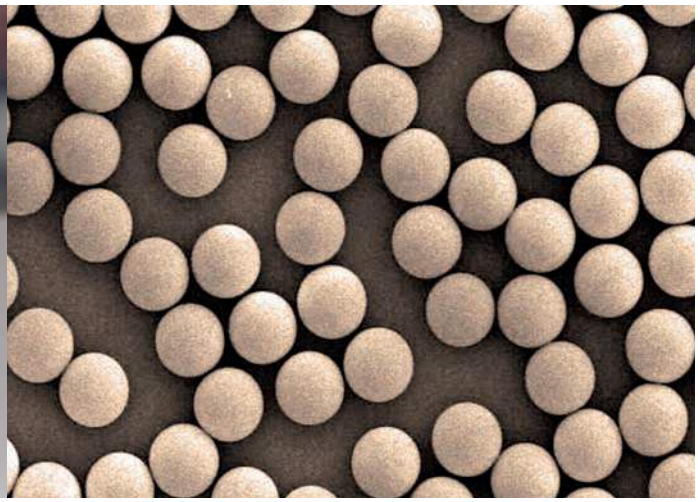
[1] Barrios-Rodiles et al (2005) Science 307:1621-5

August, 2008
Dr. Manfred Koegl
Translational Research, and Genomics and Proteomics Core Facilities, German Cancer Research Center
Im Neuenheimer Feld 515
69120 Heidelberg, Germany
www: http://www.dkfz.de/gpcf/y2h.html

395 570 V1.0, 08-2008

Efficient and gentle processing of magnetic Dynabeads®

using Tecan's HydroFlex™ microplate washer



Introduction

The use of magnetic beads as the solid phase in ELISA (Enzyme-linked immunosorbent assay) has several advantages over direct coupling to the wells. These advantages include an increased available surface area and an even distribution of beads throughout the sample providing rapid and sensitive detection of low analyte concentrations (1. Liabakk et al).

When Dynabeads® are used as the solid phase, the capture antibody is coupled to the beads in bulk, which ensures high reproducibility and eliminates the need to perform QC of each plate with antibody coupling.

Magnetic beads have thus become the gold standard for companies that provide and develop immunodiagnostic assays. By automating tedious manual wash steps, beadbased ELISAs may also get used in research labs in the future.

Using Dynabeads® in combination with the HydroFlex™ plate washer configured for magnetic bead washing, it is possible to run bead-based ELISA with the convenience of the 96-well plate format and the ease of handling known from traditional well-based ELISA.

In this application note we describe the use of Tecan's HydroFlex™ washer equipped with the smart-2 MBS magnetic carrier for automated washing of Dynabeads® in an ELISA. The model system used for this application note was a simple sandwich assay (Figure 1) designed to compare the automated processing of magnetic beads using the HydroFlex™ with the traditional manual procedure.

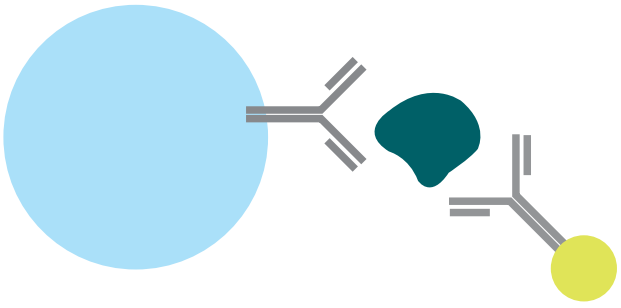


Figure 1: Schematic illustration of a bead-based ELISA. Description of model system: Dynabeads® M-280 Tosylactivated were coated with antibodies which have an affinity towards insulin. Detection of insulin was performed using a secondary antibody towards insulin, conjugated to alkaline phosphatase.

Materials and methods

Instruments

- Tecan HydroFlex™ plate washer configured with an 8-channel wash head, 4 inlet-channels for wash buffers and the optional smart-2 MBS carrier for washing of magnetic beads
- Microplate reader for detection of chemiluminescence

Dynabeads® and Microplates

- Dynabeads® M-280 Tosylactivated (Invitrogen Dynal AS, Norway)
- Greiner 96-well microplate, flat bottom, white (Greiner Bio-One, Germany, article code 655207)
- Corning® 96-well microplate, round bottom, white (Corning, US, article code 3355)

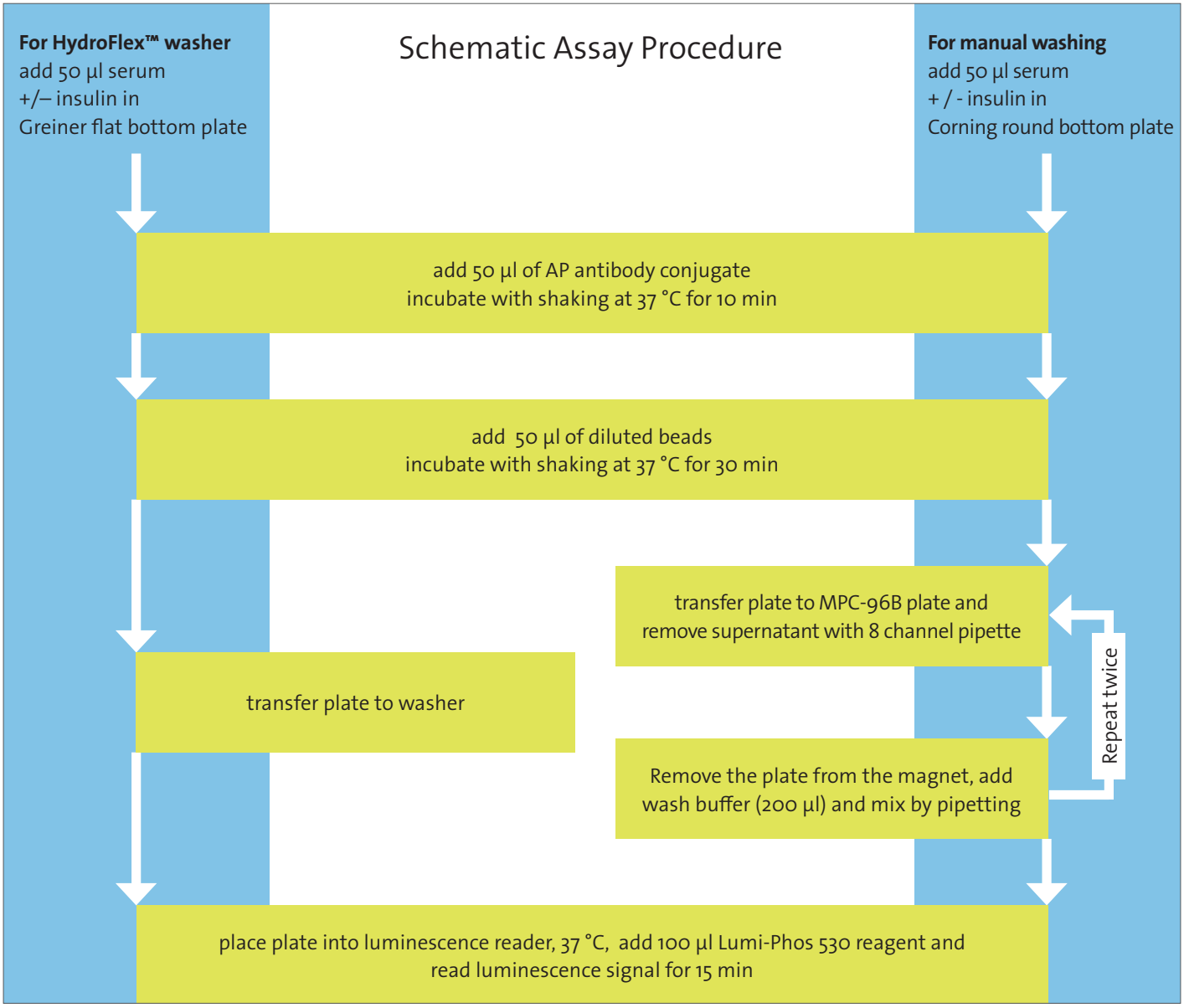


Figure 2: Schematic illustration of the experimental setup comparing the automated washing on the Tecan HydroFlex™ plate washer (left) and the manual wash procedure (right).
Due to the automation of the entire wash procedure the number of hands-on steps with the HydroFlex™ plate washer is substantially smaller compared to the manual wash procedure with a pipette.

Assay Procedure in detail

Human Insulin sandwich immunoassay:

a) Anti-insulin mAb 1 (clone 7F8, Hytest) was bound to Dynabeads® M-280 Tosylactivated in two diff erent concentrations (5 and 25 µg/mg beads) according to manufacturers instructions

b) Anti-insulin mAb 2 (clone D4B8, Hytest) was labeled with alkaline phosphatase using AbD Serotec LYNX Rapid Alkaline Phosphatase Antibody Conjugation Kit® (LNK012AP)

c) Recombinant human insulin (Sigma I2643) was dissolved in human serum (Sigma S7023) to 50 ng/ml

d) Beads with bound antibody (step a) were diluted to 0.4 mg/ml in "DELFIA® Assay Buff er" (PerkinElmer Inc., #1244-111) and incubated at roller for 30 minutes

e) I) For HydroFlex™: 50 µl serum with and without added insulin (step c) was placed in replicates of 4 in a white, fl at bottom 96 well plate (Greiner # 655207)

II) For manual: 50 µl serum with and without added insulin (step c) was placed in replicates of 4 in a white, round bottom 96-well plate (Corning # 3355)

f) 50 µl of alkaline phosphatase-antibody conjugate (step b) diluted 1:1000 (approx 5 ng/ml) in "DELFIA® Assay Buffer" was added to the wells and the plate incubated with shaking at 37°C for 10 minutes

g) 50 µl (20 µg) of the diluted beads (step d) were added to the wells and the plate incubated with shaking at 37°C for 30 minutes

h) I) For HydroFlex™: Automated washing of the plate on the HydroFlex™ using the program "Inv6_mag" and "DELFIA® Wash Buff er" (PerkinElmer Inc., #1244-114) in channel 1.

II) For manual: The plate was washed manually using a 8 channel pipette and a MPC™-96B magnet (Invitrogen Dynal AS, Norway). Three washes with "DELFIA® Wash Buff er" with resuspension of the beads at each dispensing step were performed. The plate was then left on the magnet for 30 seconds before aspirating the supernatant

i) The plate was transferred to a chemiluminescence reader pre-heated to 37 °C. 100 µl Lumi-Phos® 530 (Lumigen, Inc.) was added to the wells and the increase in luminescent signal monitored for 15 minutes

j) The slope of the curve (RLU/min) was calculated and used for comparing the samples.

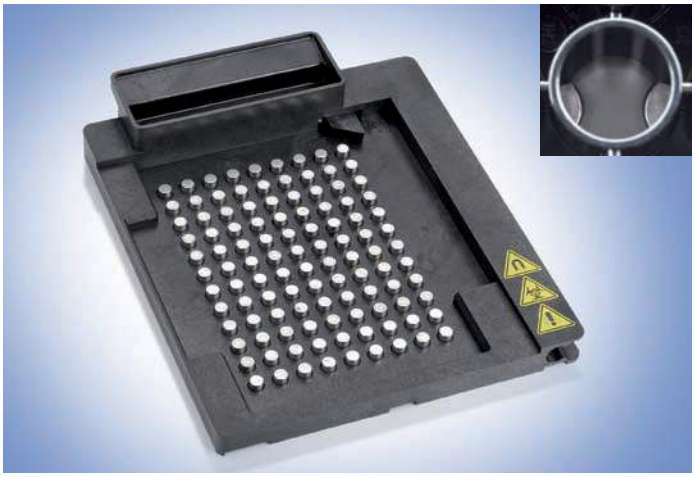


Figure 3: Optional smart-2 MBS carrier for automated washing of Dynabeads® with Tecan's HydroFlex™.

Instrument Settings

Wash Program "Inv6_mag"	Parameters
Cycle 1	**# of cycles: 1**
- Soak	60 s, shaking @ medium intens.
Cycle 2	**# of cycles: 2**
- Aspirate: z-pos. custom: 4 mm	1 s, asp. rate:1, head sp. 8 mm/s
- Dispense: z-pos. overflow	Ch.1, 200 µl, disp. rate: 300 µl/s
- Soak	60 s, shaking @ medium intens.
Cycle 3	**# of cycles: 1**
- Aspirate: z-pos. custom: 4 mm	1 s, asp. rate:1, head sp. 8 mm/s
- Dispense: z-pos. overflow	Ch.2, 200 µl, disp. rate: 300 µl/s
- Soak	60 s, shaking @ medium intens.
- Final Asp.: crosswise, z-pos. custom 6.3 mm	1 s, asp. rate:1, head sp. 8 mm/s

Table 1: Wash program tested for magnetic bead purification using the HydroFlex™ microplate washer.

Results

The chemiluminescent signals of a Dynabeads® based human Insulin sandwich Immunoassay were compared using an automated wash procedure provided by the Tecan HydroFlex™ microplate washer and a manual wash procedure via a multichannel pipette.
Additionally, the effect of two diff erent concentrations of antibodies coupled to the Dynabeads® were compared.
(see Figure 4 and Figure 5)

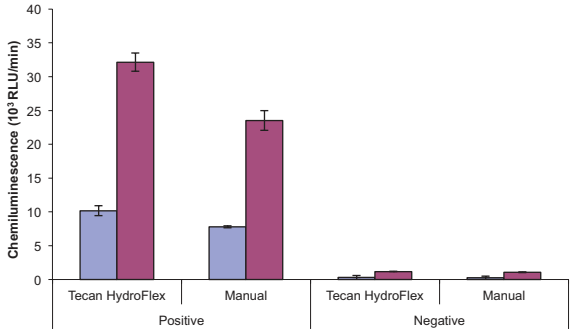


Figure 4: Chemiluminescent signals from two different concentrations (5 and 25 µg Ab/mg beads) of antibodies coupled to Dynabeads® processed automatically using Tecan's HydroFlex™ washer and processed manually. Both positive and negative controls give specific signals, due to the presence of insulin in the negative control serum.

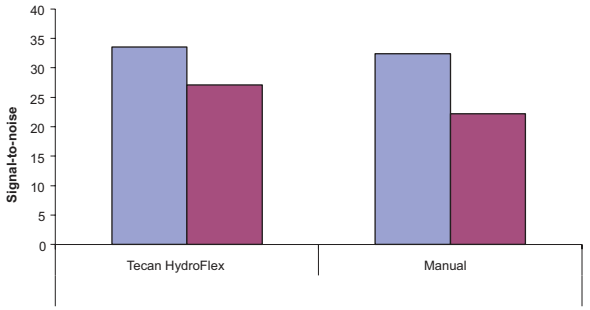


Figure 5: Signal-to-noise ratio for the two different Ab coupled Dynabeads® (5 and 25 µg Ab/mg beads) comparing the automated and manual protocol. The signal-to-noise ratio is better for the automated washing procedure using Tecan's HydroFlex™ compared to the manual washing.

Conclusion

The HydroFlex™ microplate washer was successfully used to automate time consuming wash steps of an ELISA-assay based on magnetic Dynabeads®, which up to now had to be performed manually using a multichannel pipette.
Dynabeads® in combination with the HydroFlex™ plate washer make it possible to run magnetic bead based ELISA with the convenience off ered by the 96-well plate format and the ease of handling known from traditional well based ELISA.

The results showed no signifi cant differences in the standard deviations or signal-to-noise ratios, indicating that the wash effi ciency for the automated procedure with the HydroFlex™ plate washer is as good as with the manual procedure, where the Dynabeads® beads must be fully resuspended in each wash cycle.

The signals obtained using the HydroFlex™ plate washer were slightly higher than for the manual wash procedure using a multi-channel pipette, indicating a slightly lower loss of beads (or detection antibody) during the automated wash process. This shows that the automated wash is gentle and eff ective.

Low positive values were expected for the negative samples since some insulin was present in the human serum used in this study.

Acknowledgements

We express our acknowledgements to Mr. Erling Finne and Mrs. Ingrid Manger for the assay optimization, analysis of the data and writing this note.
For more information please see:
- www.invitrogen.com/dynabeads
- www.tecan.com/HydroFlex

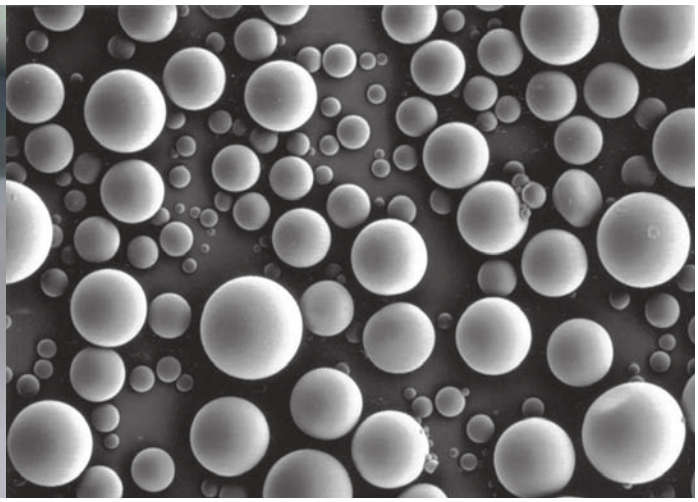
Literature

1. Nina-Beate Liabakk, Kjell Nustad, Terje Espevik (1990)
A rapid and sensitive immunoassay for tumor necrosis factor using magnetic monodisperse polymer particles. Journal of Immunological Methods, 134, 2, 253-259

396 036 V1.0, 07-2009

Efficient washing of magnetic beads

Using Tecan's HydroSpeed™ plate washer for EMD Millipore's MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel



Array of beads

Introduction

This application note describes the outcome of a successful evaluation study of Tecan's new HydroSpeed plate washer for efficient washing of magnetic beads using EMD Millipore's MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel.

Cytokines are soluble proteins acting as cell-signalling proteins to modulate the functional activities of immune cells and other tissues. Analyzing cytokines increases our understanding of the immune system and its multifaceted response to most antigens, especially those responses that make up the inflammatory process.

To identify specific cytokines involved in any inflammatory or immune response, screening panels of cytokines is of high importance. The use of magnetic beads as the solid phase in a multiplexed ELISA enables high throughput cytokine screening and offers several advantages over common ELISA assays with direct coupling of the analyte to the well-surface. These include an increased available surface area as well as an even distribution of beads throughout the sample, providing rapid and sensitive detection of low analyte concentrations (1). EMD Millipore's MILLIPLEX® MAP Human Cytokine/ Chemokine Magnetic Bead Panel enables to focus on the therapeutic potential of cytokines, as well as on the modulation of even low levels of cytokine expression. Based on the Luminex xMAP® technology in a magnetic bead format, this kit allows the quantitative, multiplexed detection of dozens of analytes in parallel, helping to increase productivity.

Tecan's HydroSpeed plate washer equipped with the optional, field-upgradeable smart-2 MBS 96 magnetic plate carrier, is an efficient solution for automated magnetic bead washing, eliminating tedious, manual dispense and aspiration steps.

The smart-2 MBS 96 magnetic plate carrier, equipped with two powerful magnets per well, helps to protect magnetic beads during washing, by effectively settling them outside of the aspiration zone (figure 2).
This patent-pending design allows optimized results to be achieved, by providing low residual volumes while maintaining high bead recovery rates.

Materials and Methods

Instruments

- HydroSpeed plate washer configured with the 96-HT wash head and the smart-2 MBS 96 magnetic carrier for magnetic bead washing in 96-well plates
- Luminex® 200™ instrument.

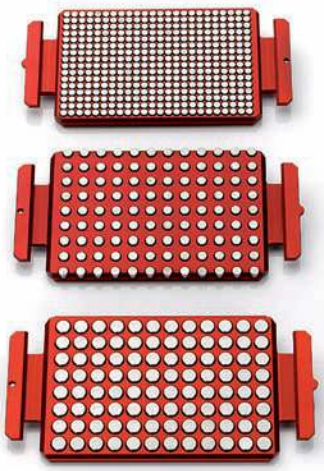


Figure 1: Selection of magnetic carriers for the HydroSpeed plate washer. From top to bottom: 384 well (MBS 384 carrier), 96 well two magnets (smart-2 MBS 96) and 96 well one magnet (MBS 96 carrier) for optimized magnetic bead washing.

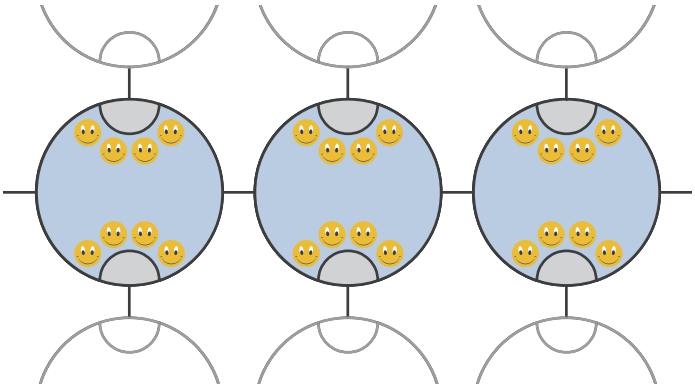


Figure 2: Schematic principle of the smart-2 MBS 96 magnetic carrier. Magnetic beads are moved to the sides of the flat-bottom wells using two powerful rare earth magnets, providing excellent bead recovery.

Microplate

- Greiner® 96-well, µclear black, flat-bottom plate (Greiner Bio-One, Germany)

Reagents

- MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel (EMD Millipore, USA)

Assay Procedure

The MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel was performed according to the assay manual (2). In an over- night incubation step at 4°C on a microplate shaker, analytes bind to the capture antibodies on the beads.

After washing with Tecan's HydroSpeed plate washer the analyte-specific biotinylated detection antibodies were added and incubated for 1 hour. During this second incubation step, the analyte-specific biotinylated detection antibodies recognize their epitopes and bind to the appropriate immobilized analyte. After removal of the free biotinylated detection antibodies, Streptavidin- conjugate was added to the fluorescent protein R-Phycoerythrin (Streptavidin-RPE) and incubated for 30 min. The Streptavidin-RPE binds to the biotinylated detection antibodies associated with the immune complexes on the beads, forming a four-member solid phase sandwich. After removing unbound Streptavidin-RPE by washing with the HydroSpeed plate washer, the beads were analyzed with the Luminex reader. By monitoring the spectral properties of the beads and the amount of associated RPhycoerythrin (RPE) fluorescence, the concentration of several analytes can be determined.

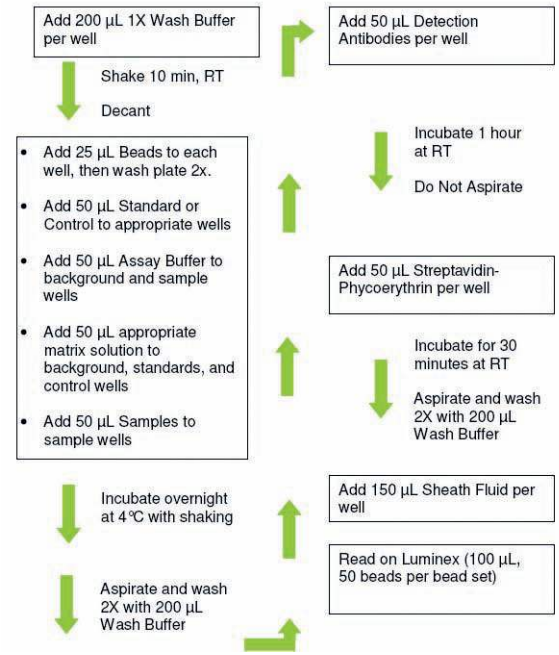


Figure 3: Workflow of kit (2).

Wash Programs

The following wash program was optimized for achieving optimal bead-recovery and a low residual volume per well.

Wash Programm	Parameters
Cycle 1	**# of cycles: 1**
Soak	90 sec
Aspirate: z-pos. Custom: 6 mm	1 sec., head speed 2 mm/s, asp. rate 1, Mode: normal
Dispense: z-pos. Overflow	Ch.1, 200 µl, disp. rate 350 µl/s
Cycle 2	**# of cycles: 1**
Soak	60 sec
Aspirate: z-pos. Custom: 6 mm	1 sec., head speed 2 mm/s, asp. rate 1, Mode: normal
Dispense: z-pos. Overflow	Ch.1, 200 µl, disp. rate 350 µl/s
Cycle 3	**# of cycles: 1**
Soak	60 sec
Aspirate: z-pos. Custom: 6 mm	1 sec., head speed 1 mm/s, asp. rate 1, Mode: normal

Table 1: Wash program for the 96-HT wash head and the smart-2 MBS 96 magnetic carrier. Residual volume after cycle 3 is approx. 13 µl.

Results and Discussion

In the first set of experiments, the bead recovery rate of the HydroSpeed plate washer equipped with the smart-2 MBS magnetic carrier was analyzed.
Using the magnetic beads contained in the MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel in combination with the wash program listed in Table 1, typical bead recovery rates of up to 97% were obtained, compared to a non-washed control.

These excellent results of the HydroSpeed plate washer demonstrate the advantage of the patent-pending design of Tecan's smart-2 MBS 96 magnetic plate carrier, using two powerful rare-earth magnets per well for fast & efficient bead settling at the side of the wells.

In the second set of experiments, the complete MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel was performed using Tecan's HydroSpeed plate washer in comparison to a typical plate washer system which was used as a "control". Table 2 and Figure 4 show the excellent correlation between the Tecan HydroSpeed plate washer and the "control" plate washer system.

Control vs. Tecan HydroSpeed plate washer		
Analyte	slope	R squared
1	0.9704	0.9771
2	0.9217	0.9779
3	0.9774	0.9774
4	0.8067	0.9682
5	1.096	0.9735
6	1.0927	0.9683
7	1.0734	0.9704
8	1.1077	0.9576
9	0.8927	0.9876
10	1.1415	0.979
11	0.9405	0.9876
12	1.0616	0.9753
13	0.9686	0.9526

Table 2: Correlation of the HydroSpeed washer to the "control" washer for the whole analyte panel.

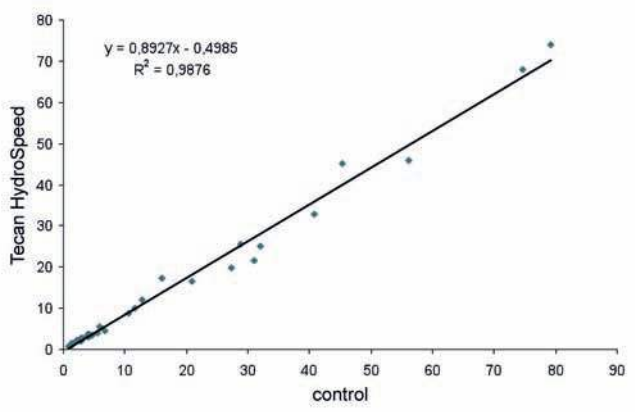


Figure 4: Representative result showing the Tecan HydroSpeed plate washer vs. the "control" for one of the13 cytokines (analyte 9).

Conclusion

The excellent wash results with EMD Millipore's MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel shown in this application note demonstrate that Tecan's HydroSpeed plate washer is an advanced solution for magnetic bead washing.

The patent-pending design of Tecan's smart-2 MBS 96 plate carrier using two extra powerfull magnets per well for efficient magnetic bead washing obtains high typical bead recovery rates of up to 97%.

The high amount of magnetic beads remaining in the wells after the wash steps with the HydroSpeed plate washer, helps to ensure fast & reliable bead counting using a typical Luminex flow cytometer system and makes Tecan's HydroSpeed plate washer the ideal solution for a range of applications using magnetic beads.

Literature

(1) Nina-Beate Liabakk, Kjell Nustad, Terje Espevik (1990), A rapid and sensitive immunoassay for tumor necrosis factor using magnetic monodisperse polymer particles. Journal of Immunological Methods, 134, 2, 253-259.
(2) MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel manual: EMD Millipore document #HSCYTMAG-60SK

List of Abbreviations

ELISA	enzyme-linked immunosorbent assay
RPE	R-Phycoerythrin

Acknowledgements

We would like to express express our thanks to Dr. Qiang Xiao R&D Manager and to Paula Grana R&D Technician from EMD Millipore in St. Charles, Missouri for their collaboration and for performing the experiments.

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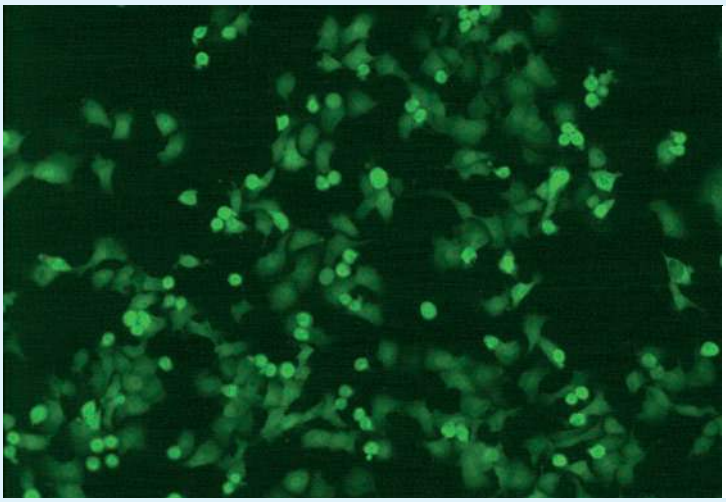
Cell washing



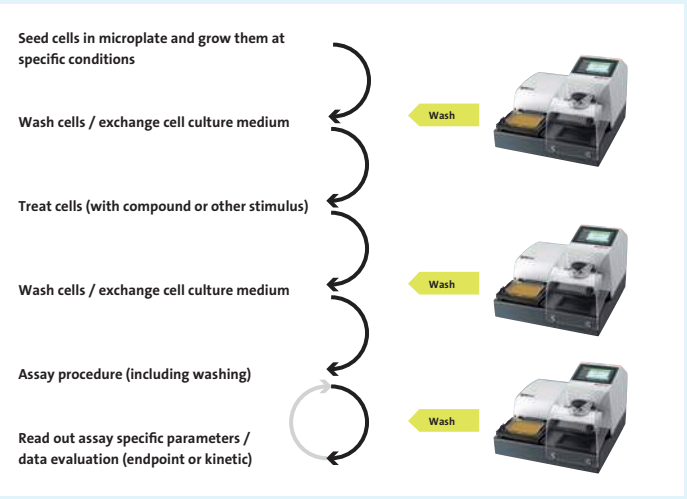


Figure 1: Common procedure for cell-based assays with living cells – washing of the microplate is an essential step in all cell-based applications.

Principle

The popularity of cell-based assays using living cells in microplate formats has dramatically increased in recent years across all segments of life science research, including academia, biotech and pharma. This is largely because the experimental results obtained from cell-based experiments better reflect the real situation invivo, than data from in vitro biochemical assays. Applications include assays for proliferation, viability, migration and cytotoxicity, as well as reporter gene assays and microbiological growth studies. All of these applications using fixed cells follow a common principle (figure 1), and include at least one washing step.

Critical parameters

Washing plates manually using standard pipettes to aspirate and dispense is monotonous and time consuming, and using vacuum-based aspiration in combination with a Pasteur pipette is typically too harsh, causing damage to the cell layer.

As discussed in the previous chapter, a lack of control of the vacuum level and the aspiration position in wells means that cells may be lost during manual wash steps and, for the same reasons, cell viability also diminishes. Combined with inter-operator variability, these limitations result in poor comparability and reproducibility of data, and restrict overall assay performance.

In theory, using a microplate washer to automate washing procedures helps to increase throughput and streamline the workload, as well as improving experimental comparability and reproducibility. However, washing plates gently enough to retain viable cells remains difficult with many commonly available plate washers, because control of critical washing parameters is limited.

Figure 2: Washing with the HydroSpeed, indicating a gentle wash performance with almost no cell detachment.

Tecan solution

As surface adherence differs between cell lines, advanced microplate cell washers such as the HydroFlex and the HydroSpeed enable direct adjustment of critical wash parameters – such as the vacuum power and aspiration position (z-position custom) within each well – allowing wash settings to be individually characterized for each cell line.

For semi-adherent or weakly adherent cells, Tecan plate washers are equipped with an extra-gentle dispense setting, allowing drop-wise dispensing to protect the cell layer from damage. A special 'Move' function also allows optimization of the wash head position relative to the rising liquid level in the wells during dispensing, minimizing cell detachment and avoiding jet effects by using the shield effect of the liquid layer above the cells to ensure good wash results.

The HydroSpeed plate washer features Cell Protection™ wash settings for maximum cell retention when working with weakly adherent cells.

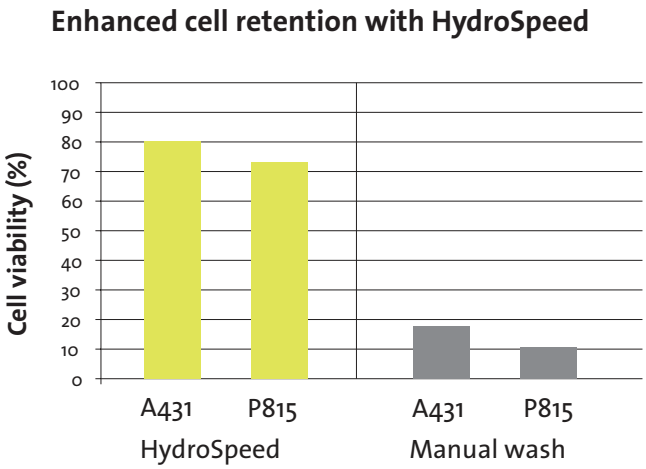


Figure 3: Comparison of cell viability as a result of optimized cell retention with the HydroSpeed plate washer compared to manual washing with a pipette

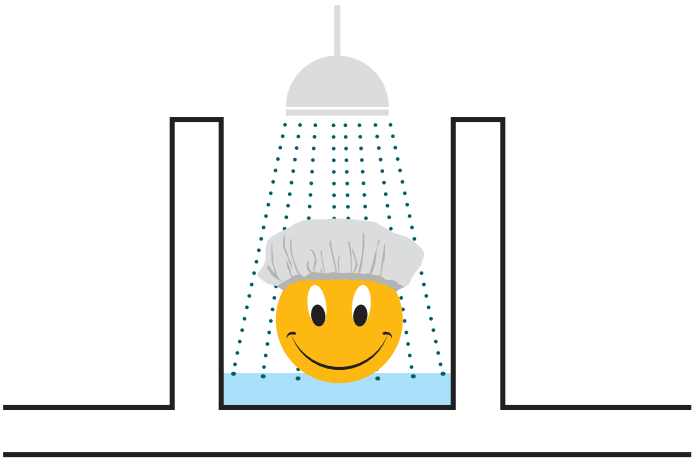


Figure 4: Extra-gentle dispense to protect the cell layer from damage.

Washer comparison for cell-based applications

Washer:	HydroFlex™	HydroSpeed™
96-well plates	x	x
384-well plates		x
Aspiration fine tuning	x	x
Dispensing fine tuning	x	x
Extra-gentle drop-wise dispense mode	x	x
Aspiration height control (z-pos. custom)	x	x
Control of aspiration rate via adjustable vacuum level and speed of wash head movement (head speed)	x	x
'Move' function to minimize dispense jet effect	x	x
Gentle Cell Protection wash settings		x
Pre-defined Rinse procedure for easy maintenance	x	x
Anti-Clogging function preventing needle blockage via automated rinsing and soaking of the wash head		x

HydroFlex and HydroSpeed: typical wash programs for gentle cell washing in the 96-well plate format

	HydroFlex™	HydroSpeed™ equipped with 96HT / 96-i wash heads
Wash program	**Parameters**	**Parameters**
96-well Cellstar® flat-bottom plate (Greiner Bio One) or similar	Polystyrene plate with special surface treatment for good adherence of cells	Polystyrene plate with special surface treatment for good adherence of cells
Plate definition file:	[GRE96ft]	[GRE96ft]
Name:	**CELL**	**CELL_96**
Cycle 1:	# of cycles: 1	# of cycles: 1
Aspirate	- Mode: normal (one asp. point / well) - z-pos: custom 8 mm - Asp. time: 1 sec - Aspirate rate: 1 - Head speed: 2 mm/s	- Mode: normal (one asp. point / well) - z-pos: custom 8 mm - Asp. time: 1 sec - Aspirate rate: 1 - Head speed: 2 mm/s
Dispense	- z-pos.: custom, 8.2 mm - move: to overflow position - disp. volume: 200 µl/well - channel: 1 - disp. rate: drip (drop-wise dispensing)	- z-pos.: custom, 8.2 mm - move: enabled - disp. volume: 200 µl/well - channel: 1 - disp. rate: 70 µl/sec (drop-wise dispensing)
Cycle 2:	# of cycles: 1	# of cycles: 1
Aspirate	- Mode: normal (one asp. point / well) - z-pos: custom 7 mm - Asp. time: 1 sec - Aspirate rate: 1 - Head speed: 1 mm/s	- Mode: normal (one asp. point / well) - z-pos: custom 7 mm - Asp. time: 1 sec - Aspirate rate: 1 - Head speed: 1 mm/s

HydroSpeed equipped with 384 HT wash head: typical program for gentle cell washing in the 384-well plate format

Wash program	Parameters	
384-well Cellstar flat-bottom plate (Greiner Bio One) or similar	Polystyrene plate with special surface treatment for good adherence of cells	
Plate definition file:	[GRE384ft]	
Name:	**CELL_384:**	
Cycle 1:	# of cycles: 1	
Aspirate	- Mode: normal (one asp. point / well) - z-pos: custom 8 mm	- Asp. time: 1 sec - Aspirate rate: 2 - Head speed: 4 mm/s
Dispense	- z-pos.: custom, 8.5 mm - move: enabled - disp. volume: 70 µl/well	- channel: 1 - disp. rate: 70 µl/sec
Cycle 2:	# of cycles: 1	
Aspirate	- Mode: normal (one asp. point/well) - z-pos: custom 7 mm	- Asp. time: 1 sec - Aspirate rate: 2 - Head speed: 4 mm/s

Typical cell-based applications and common providers

Many companies offer cell-based assays; here we mention some typical examples:

- **Promega**
  www.promega.com
  - Cell proliferation, viability and cytotoxicity assays, cell signaling assays, reporter gene systems, etc.

- **Life Technologies**
  www.lifetechnologies.com
  - Cell proliferation, viability and cytotoxicity assays, reporter gene systems (GeneBLAzer® technology), GPCR assays, nuclear receptor assays, pathway analysis assays, etc.

- **Cell Biolabs**
  www.cellbiolabs.com
  - Assays for angiogenesis, phagocytosis, cell adhesion, cell health, cell migration, cell invasion and wound healing, etc.

- **Lonza**
  www.lonza.com
  - Cell signaling, proliferation and viability assays, mycoplasma detection assays, microbial proliferation assays, etc.

- **DiscoveRX**
  www.discoverx.com
  - Cell-based assays to detect neutralizing anti-drug antibodies (ADAs), reporter gene assays and pathway analysis assays (PathHunter™), intracellular binding assays (InCELL Hunter™), cytotoxicity assays, etc.

- **Millipore**
  www.millipore.com
  - Cell toxicity assays, angiogenesis assays, stem cell osteogenesis assays, etc.

Support

Links

- Free article : Steven A Sundberg, High-throughput and ultra-high-throughput screening: solution- and cell-based approaches; Current Opinion in Biotechnology, Volume 11, Issue 1, 1 February 2000, Pages 47-53 www.molbio.gu.se/courses/drug/sundberg.pdf
- Free article : Jesús E. González et al, Cell-based assays and instrumentation for screening ion-channel targets; Drug Discovery Today, Volume 4, Issue 9, 1 September 1999, Pages 431-439 www4.ncsu.edu/~franzen/public_html/Poland/AMU_course/fluor/ion_channel_fret.pdf
- Free article : Michael V. Berridge et al, Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction; Biotechnology Annual Review, Volume 11, 2005, Pages 127-152 www.researchgate.net/publication/7548855_Tetrazolium_dyes_as_tools_in_cell_biology_new_insights_into_their_cellular_reduction/file/32bfe50e0c7cab4c24.pdf

Tecan application notes and technical notes:

- Technical note: gentle washing of cultured cells in microplates using the HydroFlex platform in drip mode; 395 065 V1.0, 05-2007
- Application note: extra gentle washing of weakly adherent cells using Tecan's HydroSpeed plate washer with Cell Protection wash settings; 396600 V1.0, 02-2011

Gentle washing of cultured cells in microplates

Using the HydroFlex™ platform in drip mode

Introduction

This technical note describes how the Tecan HydroFlex platform was successfully evaluated for gentle washing of strongly adherent cell-line A431 as well as for the weakly adherent P815 cells in a 96-well format.

Tecan's new HydroFlex platform offers advanced features for a range of applications including vacuum filtration, magnetic bead separation and washing of microplates.

For gentle cell washing, the HydroFlex platform offers individual control of critical wash parameters such as speed settings for aspiration and dispense, as well as wash head positioning, allowing fine-tuning of the wash protocol to the specific cell-type used.

A very gentle drop-wise dispense mode combined with a move function optimizes the wash head position relative to the rising liquid level in the wells, thus minimizing cell detachment and ensuring good wash results, even with weakly adherent cell types.

For the cell-based assay described in this technical note, a HydroFlex platform, equipped with a standard wash head suitable for ELISA and cell washing, was used.

For cell-based assays, the HydroFlex platform allows to remove assay reactants with no or minor influence on the viability of the used cells.

To determine the wash efficiency and viability of the cells, experiments with strongly and semi-adherent cells were performed. Additionally the cell layers were monitored before as well as after washing to visually confirm the gentle wash procedure.

Material and Methods

Instrument

- Tecan HydroFlex platform equipped with the standard 8-way manifold and two inlet channels for wash buffer

Microplates

- Greiner Bio-One 96-well (Greiner Bio-One)

Reagents and Assay Performance

Reagents

- Trypan Blue (TB), Dulbecco's Modified Eagle's Medium (DMEM, PAA Laboratories), 3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide (MTT)

Assay Protocol

Cell Culture

Human epidermoid carcinoma cells (A431, ATCC-No. CRL-1555) and mouse mastocytoma cells (P815, ATCC-No. TIB-64) were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 mM HEPES, 4 mM L-gluta-mine, 1 mM Na-pyruvate, 100 U ml-1 penicillin, 0.1 mg ml -1 streptomycin and 5 % (v/v) fetal calf serum (FCS) (all from PAA-Laboratories, Linz, Austria), in a humid atmosphere at 37° C and 7.5 % CO_2.

From the P815 cell line, adherent cells were selected by a series of washing procedures over a period of four weeks, where the supernatant (containing the suspension cells) was replaced by fresh DMEM supplemented as listed above.
For all assays, 2 x 10^4 cells of the respective cell line in 100 µl DMEM (supplemented as above) were seeded into each well of a 96-well microplate.

The wash efficiency of the HydroFlex Platform was determined by monitoring the removal of a coloured solution. Therefore, 20 µl of 0.05 % Trypan Blue (TB) was added to each well, the absorbance at 565 nm determined (= 100 %) and the washing procedure started. After washing, the re-maining TB was determined again via absorbance measurements.

In order to determine cell viability, the MTT assay was performed on cells without a washing treatment (= 100 % viability) as well as on cells with a washing treatment (= x % viability).
The MTT assay works on the principle that a metabolically active, viable cell reduces the soluble yellow tetrazolium salt MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide] by the mitochondrial dehydrogenase activity, forming an insoluble dark formazan. The resulting absorbance values from the resolved formazan can be used to determine the viability and the number of viable cells.

Wash Programs

Each program listed below was tested for the effect of different wash volumes (600 µl in Program 1 and 800 µl Program 2) and the effect of repeating wash steps (one or two wash steps) on wash efficiency and on cell viability.

Programm 1	
Wash 1x / 2x	
Z-position	10.5 mm + Move
Volume	600 µl
Head speed	1 mm / s
Wash rate	Drip mode
Aspirate	
Mode	Normal
Z-position	8500 µm
Time	1 s
Head speed	1 mm / s
Aspiration rate	1

Programm 2	
Wash 1x / 2x	
Z-position	10.5 mm + Move
Volume	800 µl
Head speed	1 mm / s
Wash rate	Drip mode
Aspirate	
Mode	Normal
Z-position	8500 µm
Time	1 s
Head speed	1 mm / s
Aspiration rate	1

All runs were performed with A431 and P815 cells, respectively. For all assays, 2 x 10^4 cells of the respective cell line in 100 µl supplemented DMEM were seeded into each well of a 96-well microplate.

Results

As an example figures 1 and 2 show light microscopic images of the weakly adherent cell line P815 & the adherent cell line A431 before and after performing Program 1 over above mentioned plates.

The images show that there are no holes within the cell layer. These results confirm that the HydroFlex platform is well suitable for gentle processing of adherent and weakly ad-herent cell lines, ensuring intact cell layers after washing.

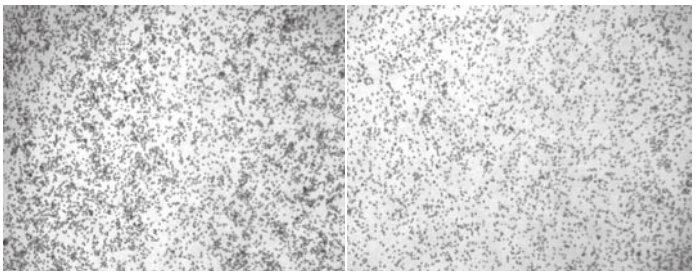


Figure 1: Weakly adherent cell line P815 before (left) and after washing (right) with PBS using Program 1

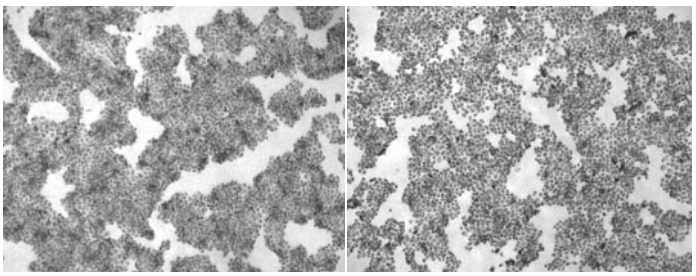


Figure 2: Adherent cell line A431 before (left) and after washing (right) with PBS using Program 1

Chart 1, below, shows the results of wash efficiency (TB-Test) and cell viability test (MTT test) after performing Program 1 or Program 2 with one or two washing steps. Each wash pro-gram was used to process two plates containing A431 and P815 cells respectively.

		Programm 1		Programm 2	
		Wash		Wash	
	µl	1x 600	2x 600	1x 800	2x 800
A431 Cells					
Wash Efficiency [%]		95.6	98.3	97.3	98.6
Cell Viability [%]		81.0	84.1	76.1	75.96
P815 Cells					
Wash Efficiency [%]		94.9	99.1	97.4	99.0
Cell Viability [%]		73.0	70.7	73.8	64.7

Chart 1: Absorbance values [%] of TB and MTT after wash procedure

Photometric evaluation shows the alliance of high wash efficiency and a minor effect on the cells by using the HydroFlex platform.

Recovered cell rate lies above 75 % with A431 cells and 70 % with P815 cells (Chart 1, above). The marginally lower recovery rates using Program 2 with 800µl volume to wash P815 cells are due to minor adhesion commonly seen with cell assays.

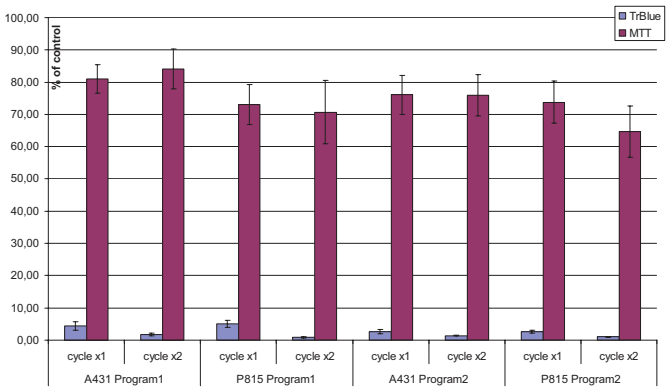


Figure 3: Content of TB and MTT after running Program 1 and Program 2 respectively.

The data obtained show high washing efficiency of about 95 % and cell viability of more than 75 % using a one step wash and aspirate program.

Conclusion

The Tecan HydroFlex platform has demonstrated excellent performance for automated washing of adherent or weakly adherent cells, combining gentle and efficient washing with very low detachment rates and good cell viability.

Advanced control of critical wash parameters such as speed settings for aspiration and dispense, as well as wash head positioning, allows easy fine-tuning of wash conditions for a range of adherent, as well as weakly adherent cell-types cultured in microplates.

Acknowledgement

We would like to thank Mag. Tobias Kiesslich, Mag. Juergen Berlanda (Research Group Prof. B. Krammer), Department of Molecular Biology, University of Salzburg
www.uni-salzburg.at/pdt · March, 2007

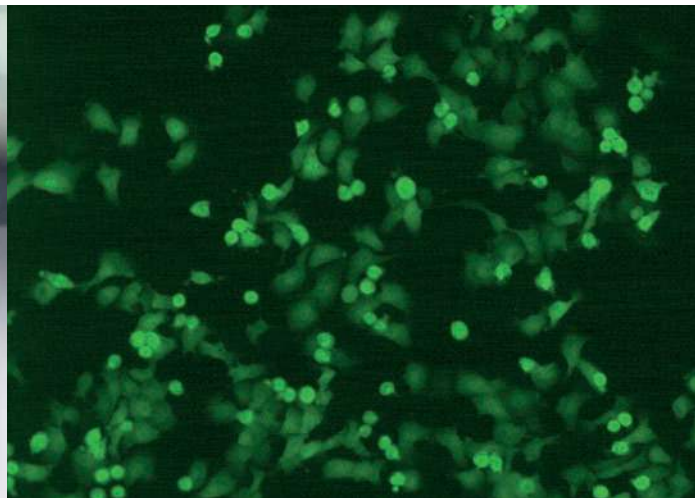
Literature

(1) Mosmann T.: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983 Dec 16; 65(1-2):55-63.
Caption

395065 V1.0, 05-2007

Extra gentle washing of weakly adherent cells

using Tecan's HydroSpeed™ plate washer with Cell Protection™ wash settings



Introduction

This application note describes the outcome of a successful evaluation study of Tecan's new HydroSpeed plate washer, using Cell Protection wash settings for extra gentle washing of adherent and weakly adherent cells in 96- and 384-well plate formats.

Tecan's new HydroSpeed plate washer offers advanced features for a range of applications including washing of cell-based assays and ELISAs, magnetic bead separation and filter washing via vacuum filtration.

The HydroSpeed provides individual control of critical wash parameters – such as the aspiration power, speed settings for gentle dispensing and wash head positioning – enabling finetuning of the wash protocol to suit the characteristics of a broad range of different cell types.

For optimized wash results, especially with weakly adherent cell types, Cell Protection wash settings offer improved cell recovery rates; combining very gentle drop-wise dispensing with fine-tuning of the dispense position relative to the rising liquid level using the Move function.

Furthermore, the HydroSpeed provides full control of the aspiration rate via tuneable vacuum settings, allowing extra gentle aspiration to remove assay reagents from the wells with little or no influence on cell viability.

To determine the wash efficiency and viability of different cell lines, experiments with adherent cells (A431) and very weakly adherent cells (P815) were performed in this study. In addition, the cell layers were monitored before and after washing to visually confirm the gentle wash procedure.

Material and Methods

Instruments

- HydroSpeed plate washer equipped with the 96 indexing wash head (96i), suitable for gentle cell washing, magnetic bead washing and ELISA washing in 96- and 384-well plates (Figure 1). High throughput wash heads for 96- and 384-well plates (96HT & 384HT) were also used for this study.
- Infinite® M200 PRO multimode reader equipped with Quad4 Monochromators™ technology

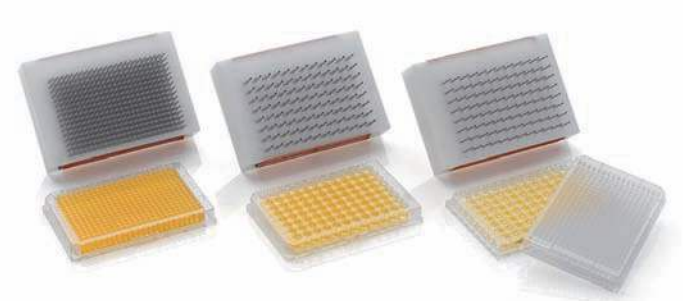


Figure 1: Wash heads available for Tecan's HydroSpeed plate washer (from left to right): high speed 384-channel wash head (384HT), dedicated 96-channel wash head (96HT) and universal 96-indexing wash head (96i)

Microplates for cell culture

- 96-well, transparent, flat bottom plate (Greiner, Bio-One)
- 384-well, transparent, flat bottom plate (Greiner, Bio-One)

Reagents

- Dulbecco's modified Eagle's medium (DMEM, PAA Laboratories)
- PBS wash buffer
- MTT (3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide)
- Trypan blue (TB)

Cell culture and assay procedures

Human epidermoid carcinoma (A431) and mouse mastocytoma (P815) cells were grown in DMEM supplemented with 10 mM HEPES, 2 mM L-glutamine, 1 mM Na-pyruvate, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 5 % (v/v) fetal calf serum (FCS) (all from PAA Laboratories, Austria) in a humid atmosphere at 37 °C and 5% CO_2.

Very weakly adherent P815 cells were selected by a series of washing procedures over a period of two weeks, where the supernatant (containing the suspended cells) was replaced by fresh DMEM supplemented as listed above.
For all assays, either 2 x 10^4 cells of each cell line in 100 µl of supplemented DMEM, or 7 x 10^3 cells in 50 µl of supplemented DMEM were seeded into 96-well and 384-well microplates respectively.

The wash efficiency of the HydroSpeed plate washer was determined by monitoring the removal of a colored solution. For this study, either 20 µl of 0.05% TB for a 96-well plate, or 10 µl of 0.05% TP for a 384-well plate were added to each well, and the absorbance at 565 nm was measured with an Infinite M200 PRO multimode reader before washing. After washing, the remaining amount of TB was determined using an absorbance measurement at 565 nm. Please note that not all P815 cells seeded into microplates become loosely adhered to the bottom of the wells. P815 cells which remain floating in the wells are typically lost during manual or automated washing, and this has been incorporated into the calculation of the cell recovery rate.
To determine the cell viability, the MTT assay was performed before and after washing. This assay works on the principle that a metabolically active, viable cell reduces the soluble yellow tetrazolium salt (MTT) to an insoluble dark formazan by the mitochondrial dehydrogenase activity. The resulting formazan can be measured by direct absorbance and used to determine the number of viable cells (1).

Cell washing using a dilution wash protocol

Dilution wash protocols consist of a sequence of aspiration and dispense steps, typically starting with an aspiration step. For optimized cell recovery, the HydroSpeed plate washer offers extra gentle settings for aspiration and dispensing steps, including the option to individually define the aspiration height for each aspiration step within a wash protocol, allowing the user to leave a defined residual volume per well.

Wash programs

The HydroSpeed wash programs used during this evaluation study were optimized to achieve both good cell viability and high wash efficiency (for details see Table 1 and Table 2).
All wash results obtained with the HydroSpeed plate washer were compared with the corresponding manual wash procedure, which used a handheld dispenser for buffer dispensing and a glass tube with an angled tip connected to a vacuum pump for aspiration. The aspiration position used for manual washing was set at the bottom of the wells, with no option for controlling the residual volume per well. The manual wash protocol consisted of a single wash cycle using either 100 µl or 50 µl of buffer for 96-well and 384-well plates respectively.

HydroSpeed wash protocol for 96-well plate format

Cells were seeded into uncoated 96-well tissue culture microplates and incubated for 16 hours at 37 oC, using 100 µl of DMEM growth medium per well.

To increase the efficiency of the first program cycle, the wash protocol started with an initial dispense step (with activated Move function) to top up the partly filled wells with 150 µl of PBS buffer prior to aspiration, as indicated in Table 1.
Extra gentle aspiration settings were used to optimize cell recovery, including an aspiration rate setting of 1 and a single point of aspiration in the centre of each well.

Wash Programm	Parameters
Cycle 1 (optional)	**# of cycles: 1**
Dispense	Custom z-pos: 8 mm with Move funct.
	Volume: 150 µl; Disp. rate: 2 (90 µl/s)
Cycle 2	**# of cycles: 2**
Aspirate	Mode: normal Custom z-pos: 6.8 mm
	Aspiration time: 1 s
	Head speed: 2 mm/s
Dispense	Custom z-pos: 7 mm with Move funct.
	Volume: 300 µl; Disp. rate: 2 (90 µl/s)
Cycle 3	**# of cycles: 1**
Aspirate	Mode: normal Custom z-pos: 8 mm
	Aspiration time: 1 s
	Head speed: 1 mm/s

Table 1: Dilution wash program for 96-well plate format using the HydroSpeed with 96i or 96HT wash heads

Wash Programm	Parameters
Cycle 1 (optional)	**# of cycles: 1**
Dispense	Custom z-pos: 7 mm with Move funct.
	Volume: 50 µl; Disp. rate: 2 (90 µl/s)
Cycle 2	**# of cycles: 3**
Aspirate	Mode: normal Custom z-pos: 8.2 mm
	Aspiration time: 1 s
	Head speed: 1 mm/s
Dispense	Custom z-pos: 8.5 mm with Move funct.
	Volume: 80 µl; Disp. rate: 2 (90 µl/s)
Cycle 3	**# of cycles: 1**
Aspirate	Mode: normal Custom z-pos: 8 mm
	Aspiration time: 1 s
	Head speed: 1 mm/s

Table 2: Dilution wash program for the HydroSpeed equipped with the 96i wash head for processing of 384-well plates

To minimize cell detachment using the 96i or 96HT wash heads, a dispense rate setting of 1 or 2 is recommended (corresponding to a dispense speed of approximately 70 µl/s to 90 µl/s).

HydroSpeed wash protocol for 384-well plate format
Cells were seeded into uncoated 384-well tissue culture microplates and incubated for 16 hours at 37 °C, using 50 µl of DMEM growth medium per well.

To increase the efficiency of the first program cycle, the wash protocol started with an initial dispense step (with activated Move function) to top up the partly filled wells with 50µl of PBS buffer prior to aspiration, as shown in Table 2.
For optimum wash results in 384-well plate formats, an aspiration rate setting of 1 and a single point aspiration per well were used.

For gentle cell washing in 384-well plates, dispense rate settings of 2 (90 µl/s) and 3 are recommended for the 96i and 384HT wash heads respectively.

Recommended wash protocols for high throughput, parallel washing of P815 and A431 cell lines using the 384HT wash head can be obtained from Tecan.

Results

Microscope images of both adherent A431 (Figure 1) and very weakly adherent P815 (Figure 2) cells were taken before and after washing, to evaluate the wash performance of the HydroSpeed plate washer.

As these images show, no holes were visible in the cell layers after washing with the HydroSpeed, indicating a gentle wash performance with almost no cell detachment.
These results confirm that the HydroSpeed plate washer is well suited to gentle processing of both adherent and weakly adherent cell lines, ensuring cell layers remain intact after washing.

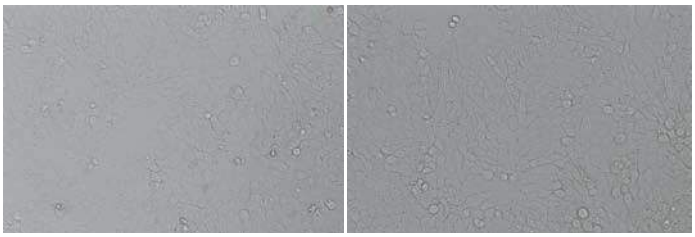


Figure 1: Adherent A431 cells before (left) and after washing with PBS (right)

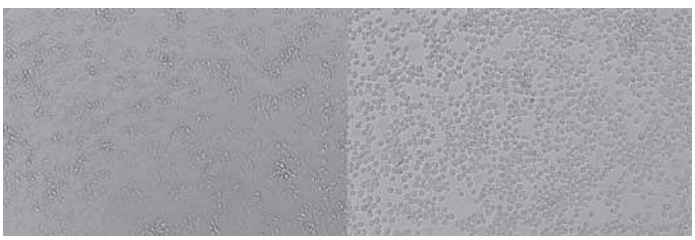


Figure 2: Very weakly adherent P815 cells before (left) and after washing with PBS (right)

Additionally, the cell viability and the wash efficiency using the HydroSpeed plate washer were compared to manual washing. Comparative results for the cell viability (MTT) and wash efficiency (TB) tests are shown in Table 3.

96-well plate washing

As shown in Table 3, the extra gentle Cell Protection wash settings of the HydroSpeed plate washer provide excellent washing performance compared to manual washing in 96-well plates.

Wash procedure	HydroSpeed plate washer with 96i head	Manual washing
A431 cells		
Cell viability [%]	80.3	18.7
Wash efficiency [%]	95.6	98.9
P815 cells		
Cell viability [%]	73.0	10.5
Wash efficiency [%]	95.4	97.3

Table 3: Comparative results for cell viability and wash efficiency of adherent (A431) and very weakly adherent (P815) cells using the HydroSpeed plate washer and manual washing

The recovery rates of viable cells after gentle and efficient washing with the HydroSpeed plate washer were nearly seven times higher for very weakly adherent P815 cells, and more than four times higher for adherent A431 cells, than after manual washing.

Results obtained with the 96HT wash head (not shown here) were comparable to the data obtained for the 96i wash head.

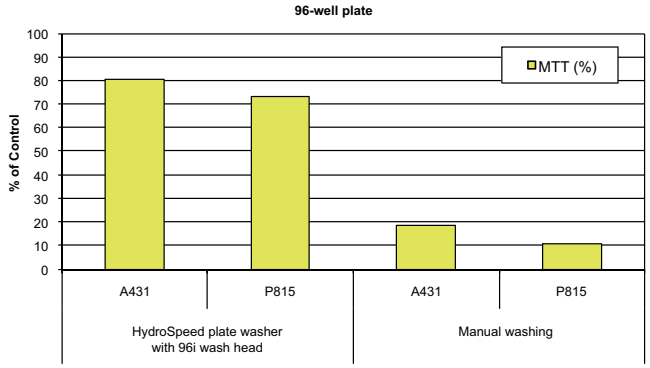


Figure 3: Comparison of cell viability (MTT) results relative to unwashed controls, obtained with the HydroSpeed plate washer (using program listed in Table 1) and manual washing in 96-well format.

384-well plate washing

As shown in Table 4, the extra gentle Cell Protection wash settings of the HydroSpeed plate washer provide excellent cell washing performance compared to manual washing techniques in 384-well plates.

Wash procedure	HydroSpeed plate washer with 96i head	Manual washing
A431 cells		
Cell viability [%]	60.8	32.0
Wash efficiency [%]	96.8	98.1
P815 cells		
Cell viability [%]	58.0	3.0
Wash efficiency [%]	93.1	98.5

Table 4: Comparison of cell viability and wash efficiency results for adherent (A431) cells and very weakly adherent (P815) cells using the HydroSpeed plate washer and manual washing

The cell viability results after gentle and efficient washing with the HydroSpeed plate washer were more than fifteen times better for very weakly adherent P815 cells, and nearly twice as high for adherent A431 cells, than after manual washing. Results obtained with the high throughput 384HT wash head (not shown here) showed viable cell recovery rates of over 77 % for both A431 and P815 cells.

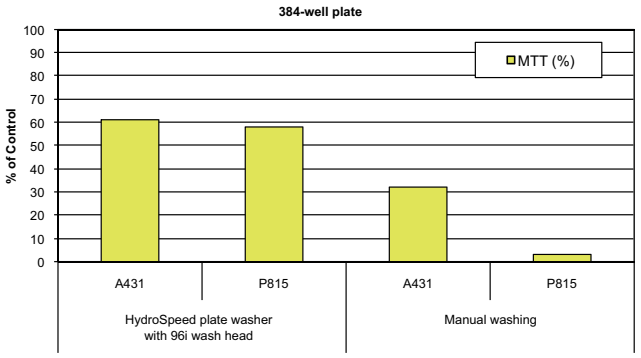


Figure 4: Comparison of cell viability (MTT) results relative to unwashed controls, obtained with the HydroSpeed plate washer (using program listed in Table 2) and manual washing in 384-well format.

Discussion

Gentle and efficient washing of weakly adherent cells is influenced by several critical wash parameters, such as the aspiration power, the aspiration height and the dispense rate.

The HydroSpeed plate washer provides advanced cell wash settings allowing the user to individually customize the aspiration height for each aspiration step. Together with the adjustable aspiration rate (aspiration power) this makes it possible to fine-tune the wash parameters of the HydroSpeed to the properties of the cell lines under evaluation.

In combination with the extra gentle drop-wise dispense speed and the Move function, the HydroSpeed plate washer provides full control of the critical wash parameters for optimized results, especially with loosely adherent cell lines.

According to the data obtained during this evaluation study, both the HydroSpeed dilution wash protocols and wash programs running in the 'overflow' position (results not shown) are highly effective for gentle cell washing in 96-well and 384-well plate formats.

The HydroSpeed plate washer provides excellent wash performance with both very weakly adherent P815 cells and adherent A431 cells, achieving good recovery of viable cells compared with manual washing.

The HydroSpeed plate washer equipped with the 96i wash head offers a very versatile solution that combines high flexibility with good wash performance for gentle washing of loosely adherent cells in 96-well and 384-well plates.
The optional 384HT wash head is recommended for high throughput applications, allowing fast, parallel washing of 384-well plates with no loss of performance for weakly adherent cells.

Conclusion

Tecan's new HydroSpeed plate washer has demonstrated excellent performance for automated washing of adherent and weakly adherent cells. It combines gentle, efficient washing with high cell retention rates and good cell viability.

The HydroSpeed plate washer provides advanced control of critical wash parameters such as aspiration rate, dispense speed and wash head position, allowing straightforward fine-tuning of wash conditions for a wide range of adherent and weakly adherent cell lines.

List of abbreviations

A431	human squamous epithelial carcinoma cells
DMEM	Dulbecco's modified Eagle's medium
FCS	fetal calf serum
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
MTT	3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide
P815	mouse mastocytoma cells
PBS	phosphate buffered saline
TB	trypan blue

Literature

(1) Mosmann T.: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays; Journal of Immunological Methods, 1983 Dec. 16; 65(1-2):55-63.

Acknowledgements

We would like to express our thanks to Univ.-Doz. Dr. Kristjan Plaetzer from the Division of Physics and Biophysics of the Department of Materials Science and Physics, University of Salzburg, as well as to Mag. Julia Knaup and Mag. Verena Ziegler (University of Salzburg) for their collaboration in providing cell cultures and performing the experiments.

Groedig, Austria, February 2011

396600 V1.0, 02-2011

Appendix

Abbreviations

A431	human epidermal carcinoma cells
DMEM	Dulbecco's modified Eagle's medium
ELISA	enzyme-linked immunosorbent assay
FCS	fetal calf serum
HBs Ag	surface antigen of Hepatitis B virus
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HT	high throughput
MT	3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide
NC	negative control
Neg	negative patient samples
OD	optical density
P815	mouse mastocytoma cells
PBS	phosphate buffered saline
PC	positive control
Pos	positive patient samples
RPE	R-Phycoerythrin
TB	trypan blue
TMB	tetramethylbenzidine

Tecan – Who we are

Tecan is a leading global provider of laboratory instruments and solutions in biopharmaceuticals, forensic, clinical diagnostics, academic centers and life science industries, and specializes in the development and production of automated solutions, detection instruments such as microplate readers, microarray-related products and washers.

Founded in Switzerland in 1980, Tecan has manufacturing, research and development sites in both North America and Europe, and maintains a sales and service network in 52 countries. To date, Tecan has distributed approximately 20,000 microplate readers worldwide and is committed to continuous technological improvements and compliance to global quality standards.

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Sweden +46 31 75 44 000 **Switzerland** +41 44 922 81 11 **UK** +44 118 9300 300 **USA** +1 919 361 5200 **Other countries** +43 62 46 89 33

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