

Efficient washing of magnetic beads

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

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.

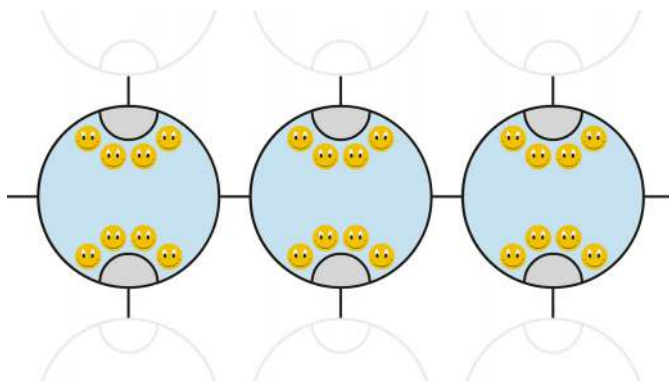


Figure 2 Schematic principle of the smart-2 MBS 96 magnetic carrier. Magnetic beads are moved to the sides of the flat-bottom wells using two powerful rare earth magnets, providing excellent bead recovery.

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 R-Phycoerythrin (RPE) fluorescence, the concentration of several analytes can be determined.

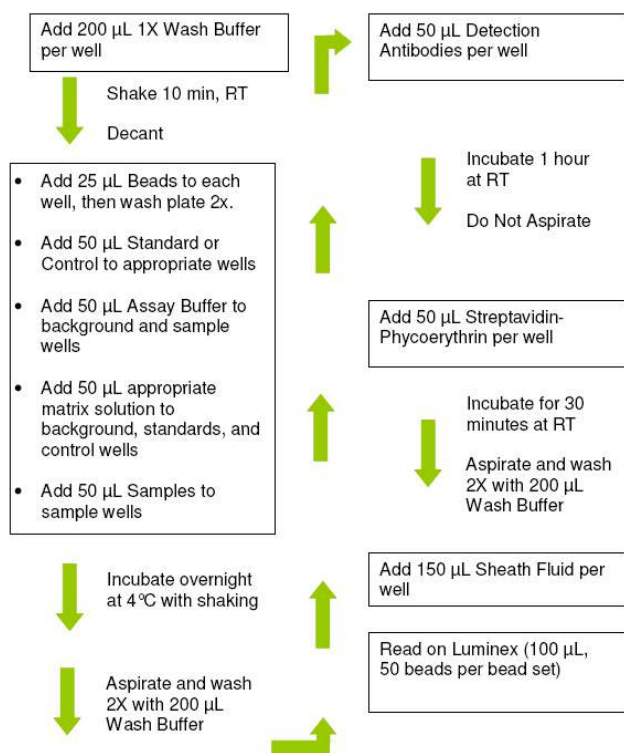


Figure 3 Workflow of kit (2).

Wash Programs

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

Wash program	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.

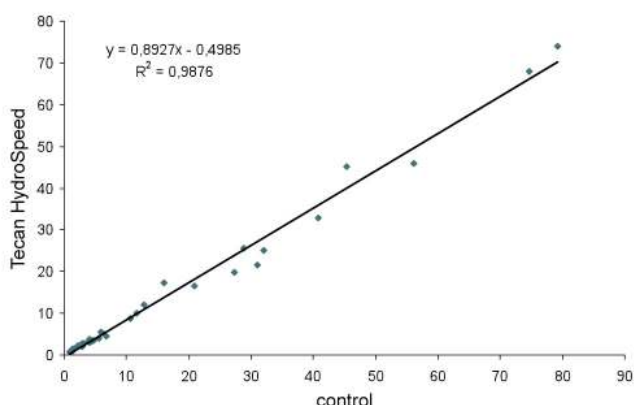


Figure 4 Representative result showing the Tecan HydroSpeed plate washer vs. the "control" for one of the 13 cytokines (analyte 9).

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 powerful 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

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