

APPLICATION NOTE

Washing MAGPIX beads with the AquaMax 2000/4000 Microplate Washer and LifeSep magnetic plate holder

Introduction

The MAGPIX multiplexing system offers high-throughput multiplexing capabilities with up to 4800 results in one hour. To ensure optimal performance of these assays, it is essential to wash the MAGPIX beads efficiently while maximizing retention of the beads within the microplate wells. With the AquaMax® 2000/4000 Microplate Washers from Molecular Devices®, bead retention of at least 95% is achievable using the optimized settings described in this document.

Materials

- MAGPIX Calibration Kit (Life Technologies, Cat. #MPX-CAL-K25)
- Phosphate buffered saline (PBS)
- LifeSep 96F magnetic plate holder (Dexter Magnetic Technologies, Cat. #2501008-1)
- 96-well clear microplates (Greiner)
- ImageXpress® Velos™ Scanning Cytometer (Molecular Devices)

Methods

Step 1. Diluted beads 1:50 in PBS and pipetted 100 µL beads into each well of a 96-well microplate.

Step 2. Read the microplate on the ImageXpress Velos Cytometer prior to washing to obtain the starting number of beads.

Step 3. AquaMax Washer microplate setup: Created a new plate in the on-board software. Started with 96-well Greiner plate, entered height as 24.2 mm (regular plate height + 10 mm LifeSep adapter height). Used the Well Depth Auto feature to determine the precise well depth and saved the resulting value in the plate definition.

Step 4. Washed plate with PBS on the AquaMax 4000 Washer. The following parameters gave the best results (minimal loss of beads during wash):

Aspirate

- Position: Edge
- Rate: Medium
- Descent speed: 3
- Height: 5 mm

Dispense

- Volume: 150 mL
- Rate: 5
- Repeat 2X from step 1.
- Aspirate (settings as above)

With the ImageXpress Velos Scanning Cytometer, fluorescence was scanned using the 532 nm laser. The number of objects detected per well before and after washing was determined using the ImageXpress Velos Cytometer software.

Benefits

- Optimized washing for high-throughput bead-based multiplexing
- Excellent retention of washed beads
- Highly adjustable wash settings for maximum flexibility



Figure 1. AquaMax 4000 Microplate Washer using the MAGPIX multiplexing system and a LifeSep magnetic plate holder.

Pre-wash bead counts for 96-well microplate

Average number of beads per well = 615

	1	2	3	4	5	6	7	8	9	10	11	12
A	603	618	639	607	588	642	586	536	612	610	626	587
B	643	652	641	623	630	607	574	657	621	623	622	636
C	621	585	650	598	638	550	604	632	621	577	586	605
D	592	607	633	595	633	620	592	633	621	622	578	591
E	589	651	635	648	629	617	631	634	610	590	610	610
F	577	596	628	574	598	575	643	623	615	602	602	546
G	639	602	600	645	682	608	585	622	600	652	600	596
H	658	634	635	642	643	643	629	647	622	604	641	628

Post-Wash bead counts for 96-well microplate

Average number of beads per well = 585

% Retention = 95.1

	1	2	3	4	5	6	7	8	9	10	11	12
A	584	572	599	597	580	621	554	552	608	605	566	515
B	634	630	643	548	622	579	608	613	535	540	598	616
C	563	590	641	591	602	575	560	594	622	533	576	554
D	495	593	568	513	605	600	587	551	573	577	499	568
E	527	618	607	634	578	593	605	604	640	575	479	539
F	575	540	621	576	595	554	607	605	617	603	574	586
G	544	577	561	567	630	621	625	595	594	626	571	506
H	608	606	546	616	625	599	628	625	611	566	599	613

Conclusion

A high rate of bead retention is essential for successful MAGPIX multiplexing assays. When MAGPIX beads were washed in a 96-well microplate on the AquaMax 4000 Microplate Washer, using the LifeSep magnetic plate holder and optimized settings as described above, 95% of beads were retained in the wells. The AquaMax Washers' highly adjustable settings and interchangeable wash heads offer the ultimate in flexibility and optimization for any assay.

Contact Us

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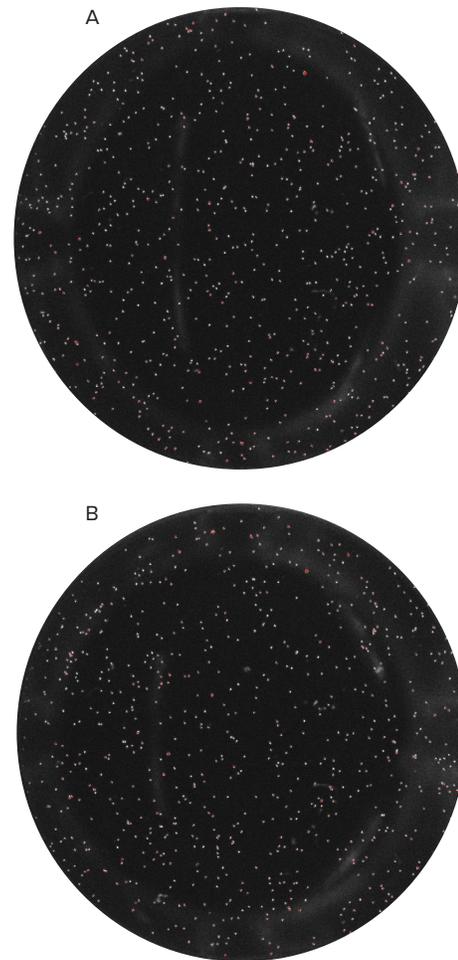


Figure 2. Images of pre- and post-wash representative wells. A. Pre-wash representative well: d5, 633 objects (beads) counted. **B.** Post-wash representative well: D5, 605 objects (beads) counted.