

Critical Automated Washer Parameters for Optimal Washing and Bead Recovery of MagPlex and MicroPlex Microspheres



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Introduction

A critical element of the use of Luminex® xMAP® microspheres (both MagPlex and MicroPlex) is the adequate removal of unbound materials from the beads during the assay procedure. With increased throughput demands, the use of automated washers such as the ELx50™ strip washer and the ELx405™ and EL406™ full plate washers has been employed. In order for an adequate wash process to take place, while at the same time providing for sufficient bead recovery to allow measurement determinations to be timely and statistically valid, the configuration of the washer settings is critical. Washing MicroPlex beads requires the use of vacuum aspiration, which can be problematic to automate due to variability of the filter plates and the starting sample matrix. Automated washing of MagPlex beads, which can be immobilized with magnets, is much less problematic provided that the proper washer settings are employed. The spatial relationship between the localization of the magnetic beads by the magnet and the aspiration tubes is paramount for bead recovery. Here we describe the washer parameters associated with low residual volumes, good bead recovery and optimal washing of microspheres. An antibody-based assay for thyroid stimulation hormone (TSH) was used to assess the ELx50 washer's ability to wash Microplex polystyrene beads by vacuum filtration. An Avian Antibodies assay developed by Luminex was used to demonstrate the utility of the ELx405 washer to automate the necessary wash steps of an antibody-based quantitative MagPlex bead assay via biomagnetic separation.

Avian Antibodies Assay Workflow - MagPlex

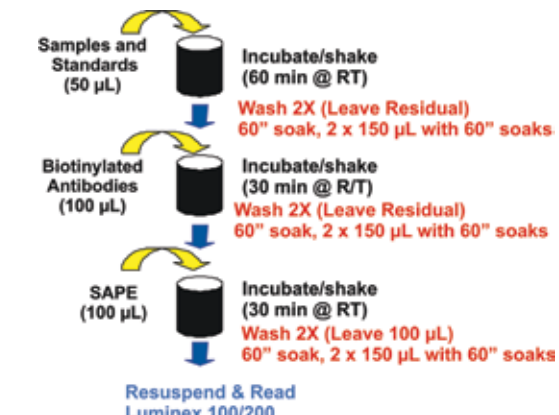


Figure 1 – Avian Antibodies Assay Protocol.

The Avian Antibodies assay protocol typifies an antibody based Luminex assay. Samples and standards (50 µL), along with 50 µL MagPlex beads were pipetted into wells of the assay microplate. The reaction was allowed to incubate for 1 hour at room temperature (RT) with agitation on a plate shaker. After incubation, the plate was washed using an ELx405 Microplate Washer equipped for biomagnetic separation (BioTek Instruments, Winooski, VT) as described in the washing instructions below. After washing, 100 µL of biotinylated secondary antibody reagent was added and allowed to incubate for 30 minutes at RT with agitation, followed by the addition of 50 µL of SAPE reagent. After a 30 minute incubation with agitation to allow for reporter tag binding to occur, the plate was again washed as described in the washing instructions, except that 100 µL of sheath fluid residual was left. Samples were then read on a Luminex 100/200™ reader (Luminex Corp., Austin, TX) controlled with XPONENT™ software (Figure 1).

Wash Process Speed - MagPlex

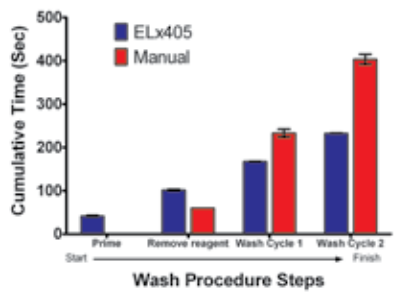


Figure 2 – Time comparison of automated and manual magnetic wash processes.

The time required for the process steps of a two cycle wash performed manually or with the ELx405 was recorded and the cumulative time plotted. The ELx405 washer consistently required 232 seconds (3 min 52 sec) to prime itself, aspirate the existing reagent and perform two cycles of a 150 µL wash with final aspiration. Using the manual flick method of magnetic bead washing was more variable, but on average required 403 seconds (6 min 43 sec) to accomplish the same tasks. Both methods utilized an initial 60 second exposure to the magnet for bead capture prior to reagent aspiration as well as 60 second capture steps before wash cycle aspirations.

Bead Recovery - MagPlex

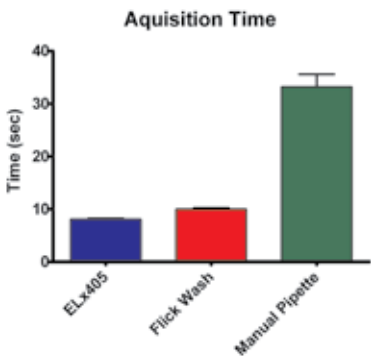


Figure 3 – Acquisition time using different wash methods.

Bead recovery after wash steps was assessed using the read step acquisition times. The amount of time necessary to acquire 50 beads of each bead type is inversely proportional to the number of beads present in the sample. The Luminex reader required an average of 8.1 seconds to interpret 50 beads each of two different bead analytes of an Avian Antibody assay when the samples were washed using the ELx405. Using the manual flick method, the same samples required an average of 10.2 seconds and 33.2 seconds when fluids were removed using a pipette.

Wash Efficiency - MagPlex Beads

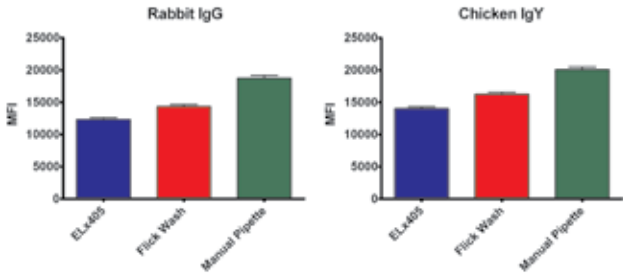


Figure 4 – Median fluorescent signal of Avian Antibody assay with different wash methods.

The same serum samples were assayed in parallel using three different means to wash the magnetic microspheres. Beads were washed using either an ELx405 with a Dexter LifeSep™ flat magnet (Dexter Magnetic Technologies, Elk Grove Village, IL), manually using a wrist flick method to remove fluid in conjunction with a Dexter magnet similar to that used by the ELx405, or using a pipette to remove wash fluids. Both analytes are prone to high amounts of non-specific binding that results in an elevated median fluorescent intensity (MFI). Efficient washing is indicated by a reduction in MFI values.

Data Repeatability - MagPlex

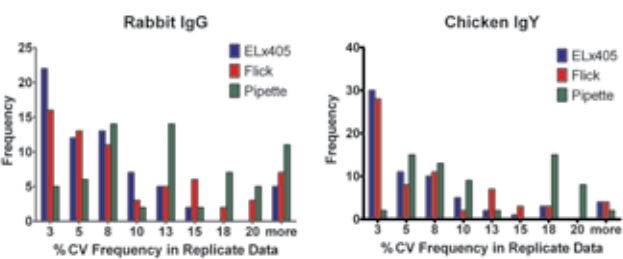


Figure 5 – Histogram depicting the frequency of sample data points with CVs depicted on the abscissa.

Comparisons were made with using magnetic beads in conjunction with the ELx405 washer, a manual flick method with a similar magnet and a pipette method the removal of fluids. When the precision of the replicate data for each of the three cases was analyzed in a histogram, it was noted that the ELx405 produced significantly higher precision and was not prone to high variance data indicative to loss of beads.

Washer Parameters - MagPlex

Washer Parameters		
File Type:	Soak	Wash
Method:		
Wash Buffer		A
Plate Type		96
Number of Cycles		2
Soak/Shake		Yes
Soak Duration	60	60
Dispense:		
Volume		150
Flow Rate		9
Dispense Height		128
Horizontal Dispense Position		0
Horizontal Y Position		0
Aspiration:		
Aspiration Height		58
Horizontal Aspiration Position		-50
Horizontal Y Position		0
Aspiration Rate		7
Aspiration Delay		0
Final Aspiration		Yes
Final Aspiration Delay		00

Table 1 – ELx405 Washer Parameters for Magnetic Bead Washing.

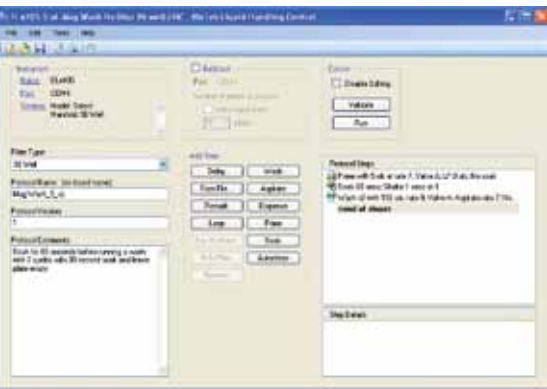


Figure 6 – LHC™ Bead Wash Program.

The ELx405 washer can be programmed via its keypad and the automated program performed as a "Link" routine that combines an initial delay for bead capture along with a wash routine. Alternatively, Liquid Handling Control™ (LHC) PC software can be used to control all washer functions remotely.

Vacuum Wash - MicroPlex

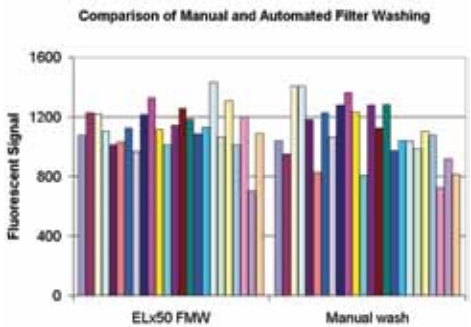


Figure 7 – Comparison of fluorescent output of manual and automated wash procedures. Equivalent TSH samples (0.015 µU/mL) were assayed in parallel using either an ELx50 washer or a manual vacuum manifold to wash MicroPlex polystyrene beads in an antibody-based Luminex assay. Data represents results from individual replicate samples. The fluorescent signal returned by the Luminex reader in both cases was compared.

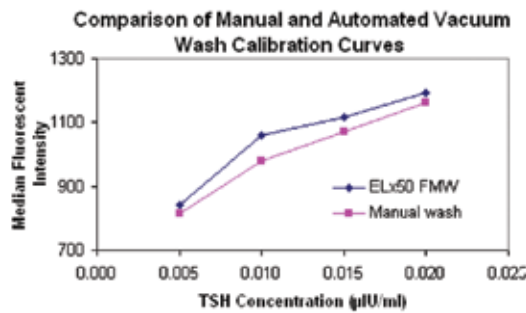


Figure 8 – Comparison of manual and automated concentration curves. Quantitative curves generated from identical TSH standards are very similar in both signal intensity values as well as curve shape.

Aliquots of samples ranging from 0.005 to 0.020 µU/mL TSH were pipetted into all wells of a 0.45 µm membrane plate (Millipore Corporation, Billerica, MA) along with PBS-4% BSA and TSH-antibody coated MicroPlex beads (2 x 10⁵ beads/mL). The TSH assay was performed with samples washed either manually or using an ELx50 washer equipped for vacuum filtration. An equivalent procedure was performed manually using a Millipore MultiScreen® vacuum manifold. After washing, 100 µL of PBS-1% BSA was added to each well and the samples were read using the Luminex reader.

BioTek Automated Vacuum Washer				
AVG	4	5	7	8
STD	0.9	0.8	1.0	0.9
% CV	20.38	14.48	15.81	11.91
Manual Vacuum Manifold				
AVG	5	7	8	9
STD	1.0	1.5	1.6	2.2
% CV	20.48	23.68	20.62	24.02

Table 2 – Comparison of Median Fluorescent Intensities.

The left column (yellow shading) represents background signals while the other columns represent incrementally increasing concentrations of TSH analyte. These data represent 5 plates of data for the ELx50 washer and 3 plates of data for the manual vacuum manifold. These data indicate that the ELx50 provides increased assay precision as compared to the manual vacuum method.

BioTek Instrumentation



Figure 9 – ELx405 Microplate Bead Washer.



Figure 10 – ELx50 Microplate Bead Washer.

Conclusions

ELx405 Microplate Bead Washer provides improved performance relative to manual methods using MagPlex beads:

- An automated sample processor for xMAP assays
- More rapid sample processing
- Greater wash efficiency
- Improved bead recovery
- Greater assay precision

ELx50 Bead Washer provides improved performance relative to manual methods using MicroPlex beads:

- Improved ease of use (no blotting of filter membranes after filtration, no clogged wells)
- Equivalent MFI
- Greater assay precision

