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Manual Washing Procedure for MagPlex Microspheres

Technical Notes

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Manual Washing Procedure for MagPlex® Microspheres

A Manual Method to Remove Liquid with the Luminex Magnetic Plate Separator

Introduction

Magnetic Microspheres are used in several methods such as, but not limited to: protein purification, protein immunoprecipitation, high throughput DNA isolation, poly (A) mRNA separation, cell separation and cell purification. Magnetic microspheres are even used in biomedical applications such as drug delivery (Saiyed, et al; 2003). The Luminex MagPlex® Microspheres can be used for multiplexed protein and nucleic acid detection using the MAGPIX®, Luminex® 100/ 200™and FLEXMAP 3D® instrument systems.

Magnetic Microspheres are typically composed of superparamagnetic material embedded within a plastic bead of 1-7 µm in diameter and are easily magnetized with an external magnetic field. Once the magnet is removed, the magnetic microspheres are immediately redispersed (Saiyed, et al; 2003). Due to these properties, magnetic microspheres have become a popular alternative to standard separation techniques, such as manual or automated filtration through a membrane. The MagPlex Microspheres are polystyrene beads embedded with superparamagnetic material measuring 6.4 µm in diameter. The functional carboxyl groups on the surface of the MagPlex Microspheres allow for easy coupling to an amine group such as those found in proteins and modified oligonucleotides. MagPlex Microspheres also contain an internal array of up to 3 dyes which color code the beads, thus allowing for up to 50-plex muliplexing using the MAGPIX, 80-plex multiplexing using the Luminex 100/200 instrument or up to 500-plex multiplexing using the FLEXMAP 3D instrument.

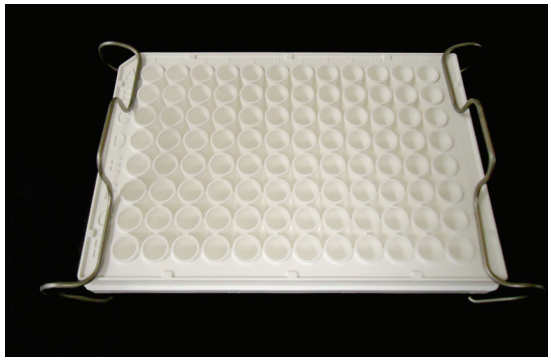
This paper describes a unique technique for manually washing a 96-well plate containing magnetic microspheres without the use of an automated plate washer or a handheld pipettor. When automation is not available, this manual liquid reagent evacuation method effectively removes supernatant and unbound analytes. Assay Medium Flourescence Intensity (MFI), bead recovery and bead carry-over are demonstrated to be within acceptable ranges when this evacuation method is utilized.

Materials

The materials used in the development of this process are MagPlex Microspheres manufactured by Luminex Corp, Austin, TX part numbers MC10XXX-01 or MC10XXX-04 where the XXX represents the bead region; the Luminex Magnetic Plate Separator; part number CN-0269-01, and white, 96 well, round bottom plate manufactured by Costar®; part numbers 3789.

Method

The assay plate is placed on the Luminex Magnetic Plate Separator at the end of an assay incubation step and sits on the separator for one minute to bring all MagPlex Microspheres to the bottom of each well (Images 1 & 2).



Images 1 & 2. Place the plate on the separator for 1 minute.

Clip the 96-well plate on the Luminex Magnetic Plate Separator, both the plate and the magnetic plate separator are rapidly and forcefully inverted over a sink or biohazard receptacle to evacuate the liquid reagent from the wells (Images 3 & 4).

Images 3 & 4. Rapidly and forcefully invert the plate and separator.

The rapid, forceful inversion motion over the sink or biohazard receptacle must be repeated two to three times in quick succession to effectively and sufficiently remove the liquid reagent from the microspheres in each well. You may see the MagPlex

Microspheres at the bottom of the well depending upon the plex and concentration in the assay (Image 5).

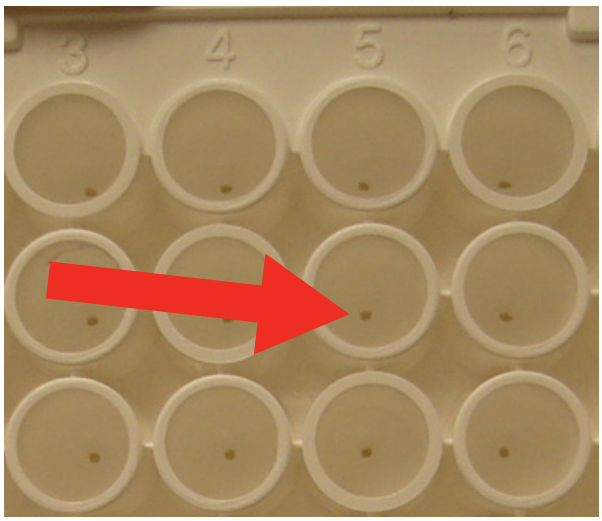


Image 5. MagPlex Microspheres may be seen at the bottom of wells following liquid evacuation.

Once the assay plate is removed from the magnetic separator, liquid reagent can be added back to each well as a part of a wash step or to proceed with the next reagent addition step in the assay protocol.

Results

Experimentation demonstrates efficient and effective evacuation of liquid reagents from the MagPlex Microspheres and assay plate wells using a rapid, forceful inversion motion to remove wash supernatant.

MFI Reporter Signal Response

The same 96 sample 6-plex assay was run twice on a Luminex 100/200 instrument with the only variant being the washing technique. Initially, 2500 beads per region were pipetted per well. The assay required 3 wash steps with 2 washes per step; therefore, 6 washes in total. One assay plate was washed using the manual method described in the Method section of this paper; and the other plate was washed using a handheld multi-channel pipettor to evacuate the supernatant from the wells.

Antibody 1 and 2 are used as positive controls for the assay. The average resulting median fluorescent intensity MFI tends to be greater using the handheld pipettor for washing the assay than using the manual washing method as seen in Table 1. The manual method is a more efficient wash step as it removes more of the unbound analyte from the well.

Manual Wash			Handheld Pipettor		
	Ab 1	Ab 2		Ab 1	Ab 2
MIN	7301	9647	MIN	7921	9532
MAX	17855	21260	MAX	24605	24585
AVG	13215	14897	AVG	17089	18457
STDEV	2649.15	2892.80	STDEV	4679.37	4463.04
%CV	20	19	%CV	27	24

Table 1. MFI Results between different methods

Recovery

Individual assay well analysis times were used as an indication of bead recovery; thus, the more beads recovered after washing steps, the quicker the well was analyzed. Typical read times can vary depending upon the number of beads used per region, the plex being analyzed, and recovery of beads after wash steps. The assay described above in the MFI Reporter Signal Response section was used to test recovery as well.

Average analysis time for the manual wash method was approximately three times faster than using a handheld pipettor, as can be seen in Table 2. This would indicate that bead recovery is three times greater on average with use of the manual washing technique described in this paper.

The variance between the minimum and maximum analysis times in Table 2 indicates that, using the manual washing method, the wells are washed more consistently. This indicates bead recovery is more consistent from well to well and can improve reproducibility. Washing is a well known source of assay variability. Better consistency in plate washing can yield better reproducibility.

Manual Wash		Handheld Pipettor	
MIN	5 seconds	MIN	7 seconds
MAX	20 seconds	MAX	97 seconds
AVG	9 seconds	AVG	29 seconds
STDEV	2.61	STDEV	20.72
%CV	29	%CV	71

Table 2. Variance of well analysis time between different methods

Carry-over

Assays based on xMAP® Technology usually experience ≤ 2% carry-over of beads into subsequent wells. The manual washing procedure described in this paper produces ≤ 2% bead carry-over as shown in the following experiment; therefore, fitting standard criteria for carry-over. Different MagPlex bead regions were pipetted into separate wells in duplicate; the plate layout is illustrated in Table 3. The plate was washed by the method described above 3 times with 2 washes each time for a total of 6 washes. Carry-over was determined by running the assay plate on a Luminex 100/200 Instrument set to detect all 10 bead regions and to collect a total of 100 bead events per well. Results demonstrate ≤ 2% bead carry-over from well to well as shown in Table 4.

	1	2	3	4	5	6	7	8	9	10	11	12
A	66	64	63	48								
B	66	64	63	48								
C	67		62									
D	67		62									
E	65		43									
F	65		43									
G	74		61									
H	74		61									

Table 3. Plate layout for bead carry-over experiment

DataType:	Count											
Location	Sample	Analyte 66	Analyte 67	Analyte 65	Analyte 74	Analyte 64	Analyte 63	Analyte 62	Analyte 43	Analyte 61	Analyte 48	Total Events
1(1,A1)	Unknown1	99	0	0	0	0	0	0	1	0	0	100
2(1,B1)	Unknown1	100	0	0	0	0	0	0	0	0	0	100
3(1,C1)	Unknown2	1	99	0	0	0	0	0	0	0	0	100
4(1,D1)	Unknown2	0	100	0	0	0	0	0	0	0	0	100
5(1,E1)	Unknown3	0	0	100	0	0	0	0	0	0	0	100
6(1,F1)	Unknown3	0	0	100	0	0	0	0	0	0	0	100
7(1,G1)	Unknown4	0	0	1	99	0	0	0	0	0	0	100
8(1,H1)	Unknown4	0	0	0	100	0	0	0	0	0	0	100
9(1,A2)	Unknown5	0	0	0	0	100	0	0	0	0	0	100
10(1,B2)	Unknown5	0	0	0	0	100	0	0	0	0	0	100
17(1,A3)	Unknown6	0	0	0	0	2	98	0	0	0	0	100
18(1,B3)	Unknown6	0	0	0	0	0	100	0	0	0	0	100
19(1,C3)	Unknown7	0	0	0	0	0	0	100	0	0	0	100
20(1,D3)	Unknown7	0	0	0	0	0	0	100	0	0	0	100
21(1,E3)	Unknown8	0	0	0	0	0	0	0	100	0	0	100
22(1,F3)	Unknown8	0	0	0	0	0	0	0	100	0	0	100
23(1,G3)	Unknown9	0	0	0	0	0	0	0	0	100	0	100
24(1,H3)	Unknown9	0	0	0	0	0	0	0	0	100	0	100
25(1,A4)	Unknown10	0	0	0	0	0	0	0	0	2	98	100
26(1,B4)	Unknown10	0	0	0	0	0	0	0	0	0	100	100

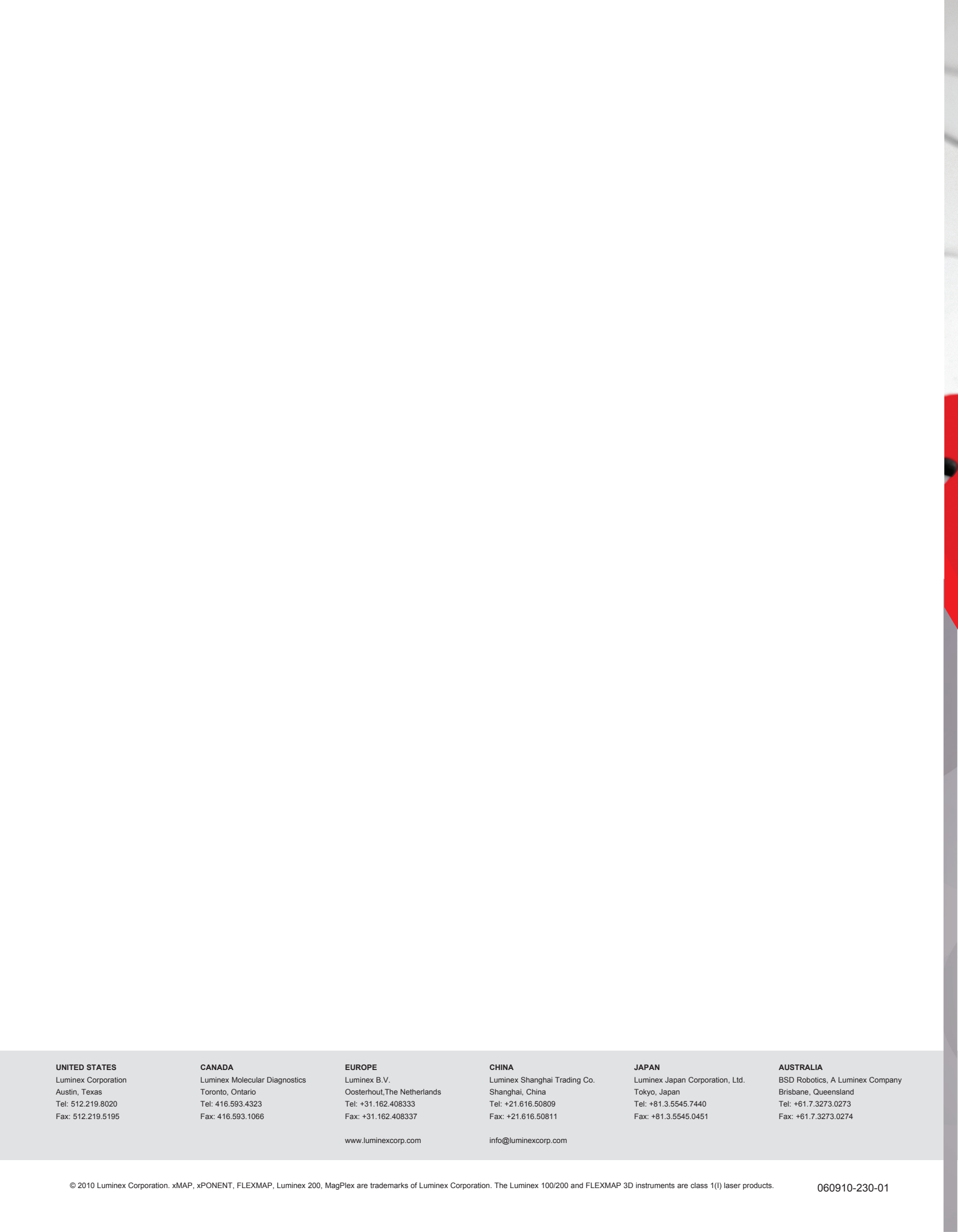
Table 4. Bead carry-over results

Conclusion

Assay MFI, bead recovery and bead carry-over are demonstrated to be within acceptable ranges when this manual liquid reagent evacuation method is utilized. When automation is not available, this manual evacuation method is as effective at removing supernatant and unbound analytes as with a handheld pipettor. This efficient method improves bead recovery and analysis read times . The manual method is ideal for low throughput labs that cannot justify the purchase of an automatic plate washer.

This method description is only intended to give guidance on how to manually remove liquid reagents with the Luminex Magnetic Plate Separator when using magnetic microspheres such as MagPlex Microspheres. It is strongly recommended that each lab validate their own technique, as user and microsphere size/ magnetic property variabilities may lead to differences in results. It is also recommended that you follow your lab's safety precautions when dealing with biological material.

Reference: Saiyed Z, Telang S, Ramchand C. "Application of magnetic techniques in the field of drug discovery and biomedicine". Biomagn Res Technol. 2003 Sep 18;1(1):2.



UNITED STATES
Luminex Corporation
Austin, Texas
Tel: 512.219.8020
Fax: 512.219.5195

CANADA
Luminex Molecular Diagnostics
Toronto, Ontario
Tel: 416.593.4323
Fax: 416.593.1066

EUROPE
Luminex B.V.
Oosterhout,The Netherlands
Tel: +31.162.408333
Fax: +31.162.408337

www.luminexcorp.com

CHINA
Luminex Shanghai Trading Co.
Shanghai, China
Tel: +21.616.50809
Fax: +21.616.50811

info@luminexcorp.com

JAPAN
Luminex Japan Corporation, Ltd.
Tokyo, Japan
Tel: +81.3.5545.7440
Fax: +81.3.5545.0451

AUSTRALIA
BSD Robotics, A Luminex Company
Brisbane, Queensland
Tel: +61.7.3273.0273
Fax: +61.7.3273.0274

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