Automated Washing of Magnetic Bead xMAP Assays for Multiplexed mRNA and Metabolic Hormone Assays

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Abstract

The next generation microsphere technology offered by Luminex is based on a partial coating of super-paramagnetic iron oxide which allows for microspheres to be immobilized under a magnetic field for easy sample processing, but still provide multiplexing capability and quantitative readouts using existing Luminex readers 100/200. Microsphere immobilization under a magnetic field considerably eases sample processing relative to the conventional filtration procedures yielding a highly automatable workflow and typically improved precision and a significant reduction in false results associated with filter well clogging. Here we demonstrate the use of a full plate magnetic microplate washer that greatly simplifies the wash cycles associated with both gene expression and protein quantitative multiplexed assays. Analytical performance with respect to bead recovery, reproducibility and quantitative results will be demonstrated.

ELx405™ Magnetic Bead Washer

- Full plate washing of magnetic microspheres
- 96- or 384-well capability
- Dual-Action™ manifold for independent control of aspiration and dispense tubes
- Allows for excellent bead recovery
- Built-in ultrasonic cleaner for easy unattended maintenance
- Automatic buffer switching for up to four wash buffers
- Choice of washer control using the built-in keypad or PC control

QuantiGene Plex 2.0 Assay and Work Flow

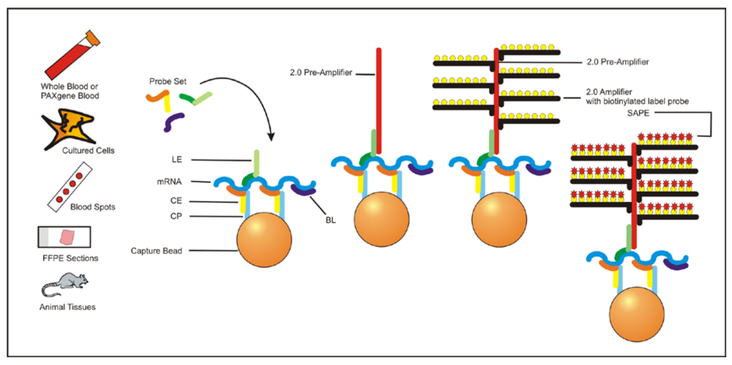
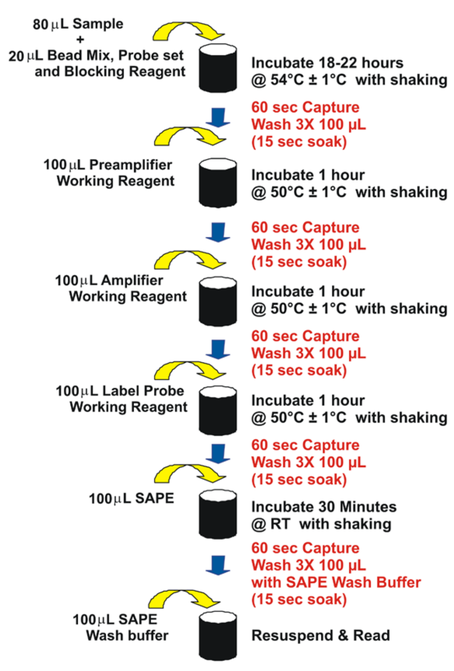


Figure 1 – QuantiGene Plex 2.0 assay principle based on the use of hybridization and branched DNA (above) and workflow (below) demonstrating the multiple wash steps using ELx405.



QuantiGene Plex Wash Performance: Recovery and Precision

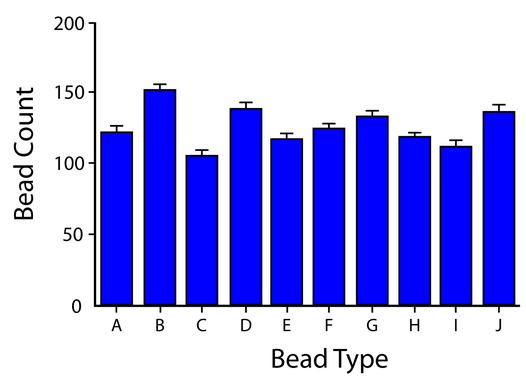


Figure 2 – Bead Recovery Studies - Bead recovery was assessed by observing the bead count of different bead types in a 10-plex assay. After adding 1000 beads of each bead type, the beads were subjected to the same reagents, wash buffers and number of washes before being read on a Luminex reader. The Luminex reader was configured to either count at least 100 beads of each type or count for a total of 45 seconds.

- Satisfactory bead recovery and precision for quantitative work.

#	Target	Mean	%CV
1	ABC B4	819	7.68
2	NFKB1	1459	7.86
3	RELA	1196	8.63
4	IFN G	1041	7.40
5	TNF	967	11.37
6	UGT1A8	917	8.64
7	UGT1A9	1520	9.34
8	ABC B11	1540	8.66
9	ACTB	1250	9.12
10	IL1B	2533	9.69
11	TNFRSF6	373	8.12
12	BAD)	1285	9.27
13	CYP2B6	1253	7.82
14	CSF2	1494	10.32
15	TNFSF6	3235	9.98
16	IL8	262	13.07
17	PPIB	1021	8.59
18	VEGF	2166	12.05
19	ABC C2	1309	8.55
20	IL10	1624	8.90
21	GAO DH)	1604	9.20
22	ABC	1664	9.45
23	CYP3A4	511	9.59
24	SLC22A7	1825	8.89
25	CYP2A6	981	9.21
26	CYP1A2)	1358	10.75
27	IL2	806	8.05
28	IL6R	1501	8.49
29	PTK2B	968	9.06
30	CDKN1A	680	9.06
31	UGT1A4	1248	9.92
32	CHUK	644	9.70
33	BCL2	962	8.02
34	UGT2B10	796	7.43
35	UBT2B7	773	11.60
36	UGT2B4	494	8.62

Table 1: QuantiGene Plex 2.0 Signal Uniformity. Using ELx405. A 36-plex assay was performed using purified hRNA species as the target. Data represent the mean and %CV of 96 determinations.

Automated Gene Expression Quantification

A 10-plex QuantiGene Plex 2.0 assay was performed on human Liver total RNA and compared to the levels in a human Universal Reference RNA sample. Data represent the mean of 4 replicates.

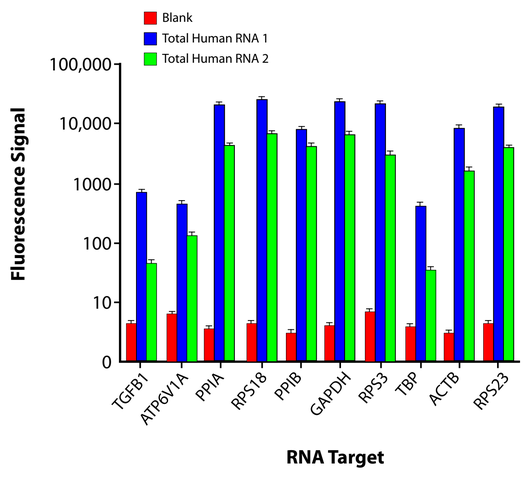


Figure 3 - Expression of RNA Species in Different RNA Samples using 96-well Reaction Plates.

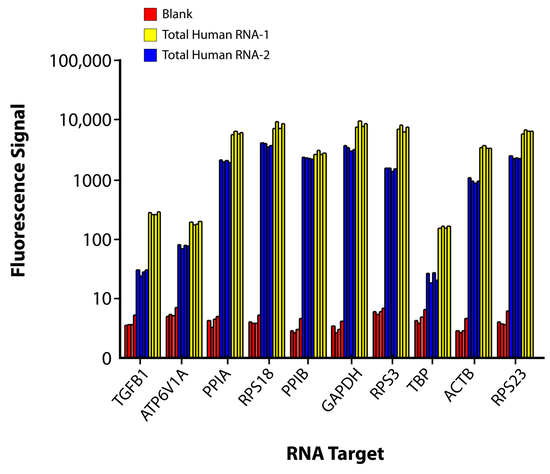


Figure 4 - Expression of RNA Species in Different RNA Samples using 384-well Reaction Plates

- Satisfactory quantitative results in both 96- and 384-well densities. Proficient washing cycles provide low background.

Human Metabolic Hormone Assay and Work Flow

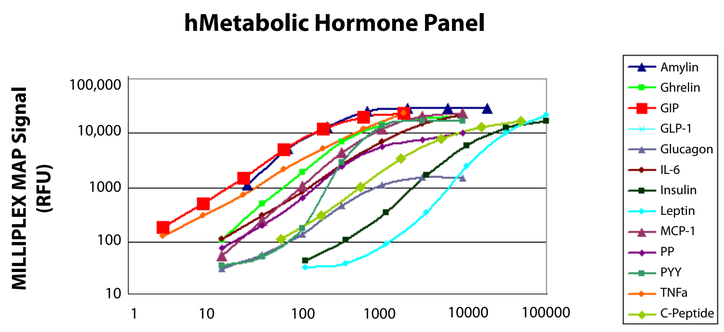
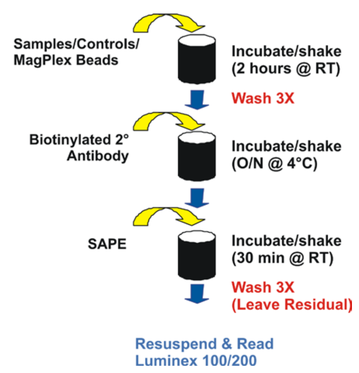


Figure 5 - Human metabolic Hormone Panel Standard Curves (above) and workflow (below) demonstrating the multiple wash steps using ELx405.



Metabolic Hormone Wash Performance: Recovery and Precision

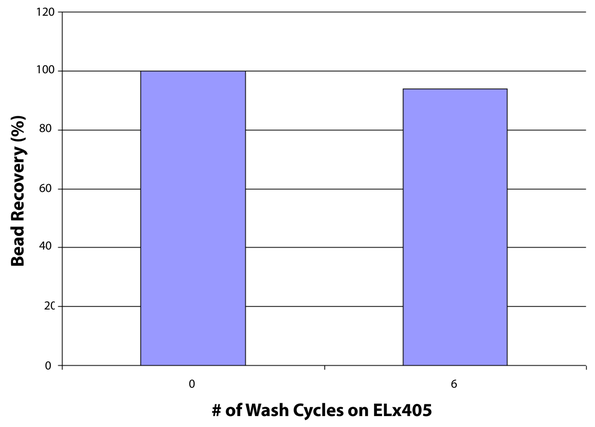


Figure 6 - Bead Recovery Studies - Bead recovery was assessed by observing the bead remaining in 96-well plate by imaging.

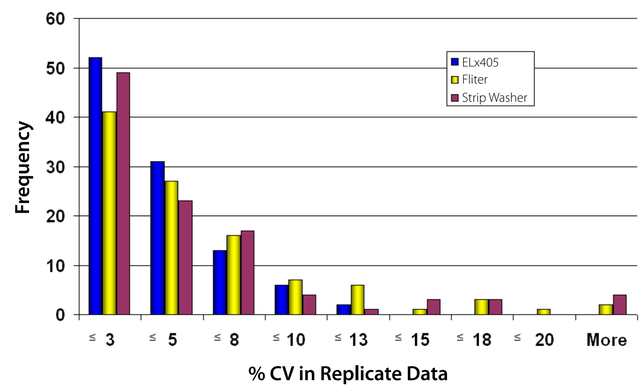


Figure 7 - Histogram demonstrating MILLIPLEX MAP data variability using same workflow but with manual filter washing, full plate washing with ELx405 and a competitor strip washer.

- Satisfactory bead recovery. Precision for ELx405 better than manual filter washing and competitive magnetic strip washer.

Conclusions

1. Bead recovery and washing efficiency satisfactory for quantitative results
   A. Gene expression assays with QuantiGene Plex 2.0 in both 96- and 384-well microplates.
   B. Protein assays with MILLIPLEX MAP human Metabolic Hormone panel in 96-well microplates.

2. Rapid sample processing with ELx405™ full plate washer.

3. Ability to integrate washer in robotic platforms