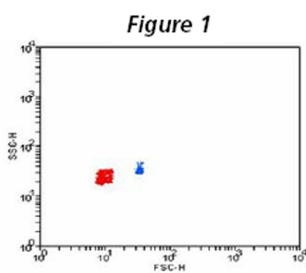




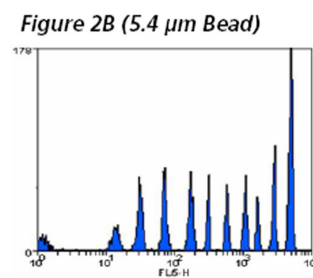
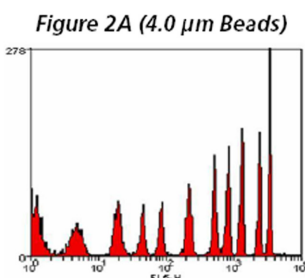
MultiBead Multiplex Assay Technology

The MultiBead assay technology relies on the ability of flow cytometers to separate beads covalently coupled to antibodies based on their size and fluorescence intensity. MultiBead assays can simultaneously quantify up to 36 analytes in a single well by utilizing multiple bead sizes each with different fluorescence intensities. Each bead size is sub-fractionated into different populations based on their inherent fluorescence intensity by a 670 nm filter, while the analytes of interest are simultaneously quantified by a 576 nm filter which measures fluorescence associated with the PE conjugated detection antibody or analyte.



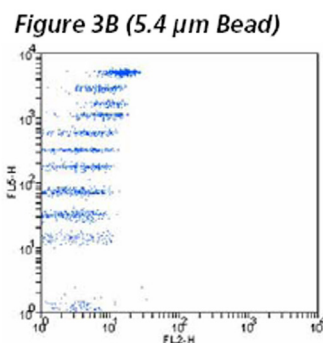
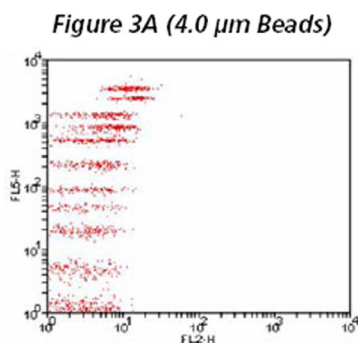
Separation of beads by size

One set of 4 μm beads was mixed with a second set of 5.4 μm beads. Each bead set was made up of 11 populations of beads having different fluorescent intensities. The two sizes of beads were analyzed in a flow cytometer and separated using forward and side scattering (Figure 1).



Separation of bead size by relative fluorescence intensity

Within each defined bead size the 11 different fluorescence intensity populations were separated in the APC channel using a 670 nm filter. This example uses the FL-5 channel to measure APC (Figures 2A-B).



Determination of amount of analyte present

The amount of each analyte is determined by measuring the fluorescence associated with a PE conjugate in the PE channel using a 576 nm filter. This example uses the FL-2 channel to measure PE (Figures 3A-B).

MultiBead Multiplex Performance and Validation

To ensure accurate results, rigorous validation experiments are performed to make certain that each bead specifically detects the analyte of interest with no interference from other antibody-bead populations or sample components (Figures 3-5).

Figure 4

		MultiBead Kit				
	IL-8	IL-1 β	IL-4	TNF- α	IFN- γ	IL-6
IL-8	4069	5.9	3.7	12.2	13.6	3.3
IL-1 β	4	1981	3	12.2	15.1	3.6
IL-4	4.5	5.5	1241	12.2	14.6	3.6
TNF- α	5	5.5	3.5	1716	14.1	3.6
IFN- γ	4.5	5.7	3.3	12.6	1946	3.6
IL-6	4.5	9.5	3.5	11.8	14.1	2996
Background	4.3	5.9	3.6	10.9	14.6	3.2
Background	4.1	5.9	2.9	12.2	13.6	3.2

Figure 4: High Specificity – 6-plex “Drop-in” Experiment. Six immunometric bead sets were multiplexed in the presence of six PE antibody conjugates. Individual cytokines (10,000 pg/mL) were then added to show that the cytokine did not bind to the other 5 antibody pairs. The 6-plex background PE fluorescence levels are shown in duplicate. The data demonstrate that the antibody pairs chosen are specific and do not cross-react with other cytokines.

Figure 5

		MultiBead Kit							
		IL-8	IL-1 β	IL-4	TNF- α	IFN- γ	IL-6	TXB ₂	PGE ₂
(A)	(-) IL-8	8.4	1844	1095	1383	1644	2839	1000	372
	(-) IL-1 β	4294	10.6	1094	1383	1855	2839	1000	382
	(-) IL-4	4538	1811	3.4	1383	1559	2892	983	393
	(-) TNF- α	4532	1812	1114	13.1	1779	2996	1037	407
	(-) IFN- γ	4454	1844	1135	1433	12.7	2739	1037	414
	(-) IL-6	4223	1844	1037	1383	1769	5.1	1018	414
(B)	(+) TXB ₂	4454	1812	1056	1383	1755	2892	430	392
	(+) PGE ₂	4372	1946	1134	1334	1638	2996	1019	98.2

Figure 5: High Specificity – “Drop-out” & “Drop-in” Experiments.

(A) Cytokine “Drop-out”: Multiplex analysis was performed with a panel of cytokines, removing one of the cytokines at a time. When each specific cytokine was removed, the corresponding fluorescence returned to background levels, demonstrating antibody specificity.

(B) Eicosanoid “Drop-in”: A duplex assay was performed with TXB2 and PGE2 antibody capture beads. Either TXB2 or PGE2 were then added to show that each eicosanoid did not bind to the other bead. In a competitive immunoassay, binding of analyte to the bead is reflected by a decrease in fluorescence.