

RayPlex[®] Human Cytokine-175 Magnetic Bead Array Kit

Quantitative Measurement of 175 Human
Proteins by Flow Cytometry

Catalog numbers:
FAHM-CYT-175-8 (8 samples)
FAHM-CYT-175-16 (16 samples)
FAHM-CYT-175-40 (40 samples)

User Manual Version 4.0
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I. Overview

Species	Human
Proteins Detected (175)	<u>Group #1 Inflammatory Cytokines:</u> Eotaxin-2, BLC, RANTES, IL-10, MCP-1, PDGF-BB, IFN-γ, TNF-α, IL-5, IL-1β, IL-6, IL-2, IL-4, G-CSF, IL-12 p70, IL-8, MIP-1α, IL-7, IL-9, TIMP-2, TNFR1, IL-1Ra, TNFR2, IL-17, I-309, IL-12 p40, IL-13, IL-15, Eotaxin-1, IL-16, MIP-1 delta (MIP-1δ), TNFβ, IL-1α, ICAM-1, IL-11, IFNα, M-CSF, GM-CSF, IL-28A, MIP-3 beta (MIP-3β), MIG <u>Group #2 Chemokines:</u> TSLP, Eotaxin-3, MIP-1 beta (MIP-1β), MDC, I-TAC, TECK, HCC-4, IL-18 Bpa, NAP-2, Osteopontin, MIF, LIF, Axl, Betacellulin, CXCL5, Lymphotactin, SDF-1α, MPIF-1, GROα, PARC, IP-10, MIP-3 alpha (MIP-3α), TARC, MCP-2, MCP-3, GCP-2, HCC-1, IL-31, IL-29, IL-17F, MCP-4, CCL28, CTACK <u>Group #3 Cytokines 1:</u> AgRP, Angiostatin, LAP, IL-2 R alpha (IL-2 Rα), Angiogenin, PDGF-AB, E-Cadherin, Activin A, Cripto-1, ICAM-2, IL-2 R beta (IL-2 Rβ), VEGFR1, Follistatin, Cathepsin S, CD40, Fcγ RIIB/C, GP130, Thrombopoietin, DAN, Galectin-7, Fas Ligand, SDF-1β, VEGF-C, TGFβ2, IL-13 R alpha 1 (IL-13 Rα1), IL-1 R4, Tie-2, TSH, Sonic Hedgehog N-Terminal (SHH-N), TREM-1, IL-23, TRAIL R4 <u>Group #4 Cytokines 2:</u> AFP, ANGPTL4, CA125, FSH, hCGβ, IGF-1R, IL-1 RII, IL-18 R beta (IL-18 Rβ), IL-21, IL-3, Leptin, MMP-10, MMP-13, MMP-3, MMP-8, NSE, OSM, Prolactin, PSA-Free, Siglec-9, TACE, TIMP-4 <u>Group #5 Receptors:</u> TRAIL R3, DR6, CD14, CEACAM-1, IL-1 R1, PECAM-1, E-Selectin, Endoglin (CD105), Trappin-2, ErbB3, CD30, IL-2 Rγ, CD80, Contactin-2, uPAR, Dtk, ALCAM, LIMP2, MICA, TIM-1, IL-17 RA, XEDAR, Fas, IL-21 R, CD40 ligand, Flt-3 ligand, GTR <u>Group #6 Growth Factors:</u> GDF-15, OPG, VEGF-D, β-NGF, Growth Hormone, IGF-1, NGFR, TGF-beta 3 (TGF-β3), BMP-4, IGFBP-2, BMP-7, VEGF-A, bFGF, TGFβ1, SCF, IGFBP-6, VEGFR3, VEGFR2, PIGF, EG-VEGF

Format	Bead-based
Detection Method	Flow Cytometry using a Flow Cytometer equipped with a blue (or green) laser (PE channel) and red laser (Alexa Fluor 700 and Alexa Fluor 647/APC channel) OR Luminex instrument
Minimal Sample Volume	25 µL per test after dilution. <i>The optimal dilution must be determined empirically by the researcher</i>
Compatible Sample Types	Serum, Plasma, Cell Culture Supernatant
Number of Replicates	At least 2 replicates. Each replicate is considered a test
Reproducibility	See Section VII (page 50) for Inter-CV and Intra-CV percentages for each protein
Assay Duration	4 hours

II. Introduction

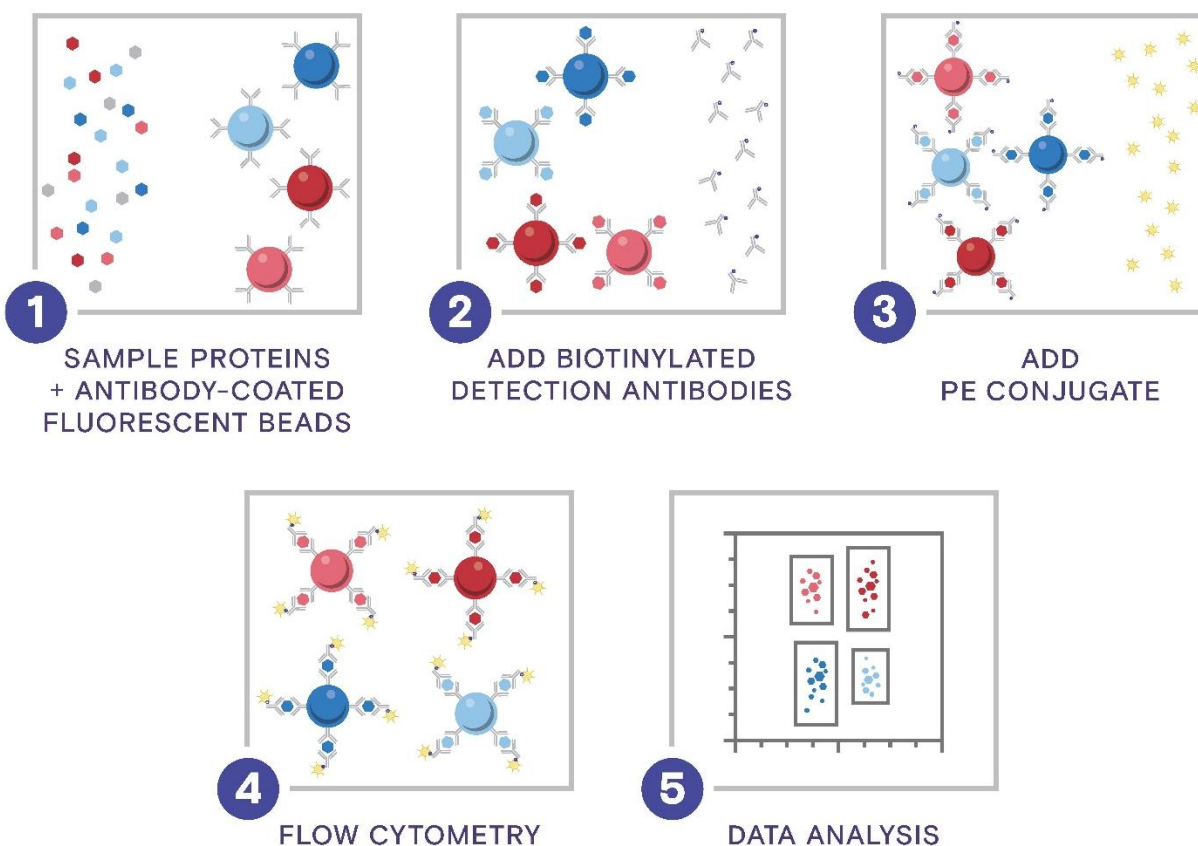
Soluble proteins play vital roles in homeostasis and disease development, including innate immunity, apoptosis, angiogenesis, cell growth, and differentiation. Thus, protein profiling has research and clinical applications, such as disease biomarker discovery and drug development. The traditional technique for detecting and quantifying soluble proteins is the enzyme-linked immunosorbent assay (ELISA), which only allows detection of one analyte each time. However, using this method to detect multiple proteins – particularly for precious samples with small quantities – becomes challenging as the processing time and required sample volume increase.

RayPlex® is a multiplexed sandwich- and bead-based quantitative antibody array for the simultaneous detection of multiple proteins using small sample volumes. It couples the versatility of RayBiotech's vast antibody pair library with familiar, reliable flow cytometry methodology. Together this creates a multiplex bead-based array requiring no dedicated instrumentation, only common flow cytometers equipped with blue (or green) and red lasers. This kit can also be used with Luminex systems.

The RayPlex® Human Cytokine-175 Magnetic Bead Array Kit is designed to assay 175 common human cytokines simultaneously. The kit uses magnetic beads that allow the use of an automatic plate washing system with magnetic separation ability during washing steps. For data in this manual, a BioTek 405 TS Washer and LifeSep™ 96F magnetic separator unit were used. Further details are included in Section VI.

III. How it Works

RayPlex® arrays are first prepared by immobilizing capture antibodies onto same size beads with dual fluorochromes and different fluorescence intensity; there is only one target's capture antibody per fluorochrome/intensity combination. The capture antibodies bind to their specific protein targets during sample incubation, and unbound proteins are removed with washing. Biotinylated detection antibodies and PE Conjugate are added, thus enabling protein detection via the sandwich immunocomplex. Individual proteins are identified by their specific bead-fluorochrome combination, while the level of PE fluorescence reflects the amount of protein that has been captured onto the beads. The protein amount can be determined (i.e., quantified) by comparing the PE signal to a standard curve generated from purified protein standards at known concentrations.



A schematic showing how RayPlex® enables multiplex protein detection with flow cytometry.

*PE Conjugate consists of PE-conjugated Streptavidin or PE-conjugated Anti-Biotin depending on kit optimization.

IV. Bead ID and Distribution

A. General View of RayPlex® Magnetic Multiplex Beads

RayPlex® Human Cytokine-175 Magnetic Bead Array Kit uses M-100 series magnetic multiplex beads which has 100 populations based on the barcode determined by the incorporated dual fluorochrome with different intensities. All the M-100 beads are equal in size. This kit uses 49 populations out of 100. (Figures 1 and 2).

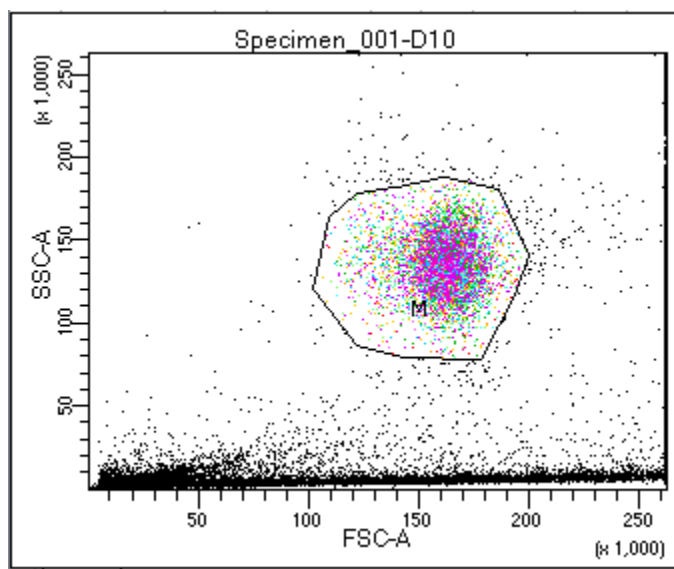


Figure 1. RayPlex® M100 beads are the same size.

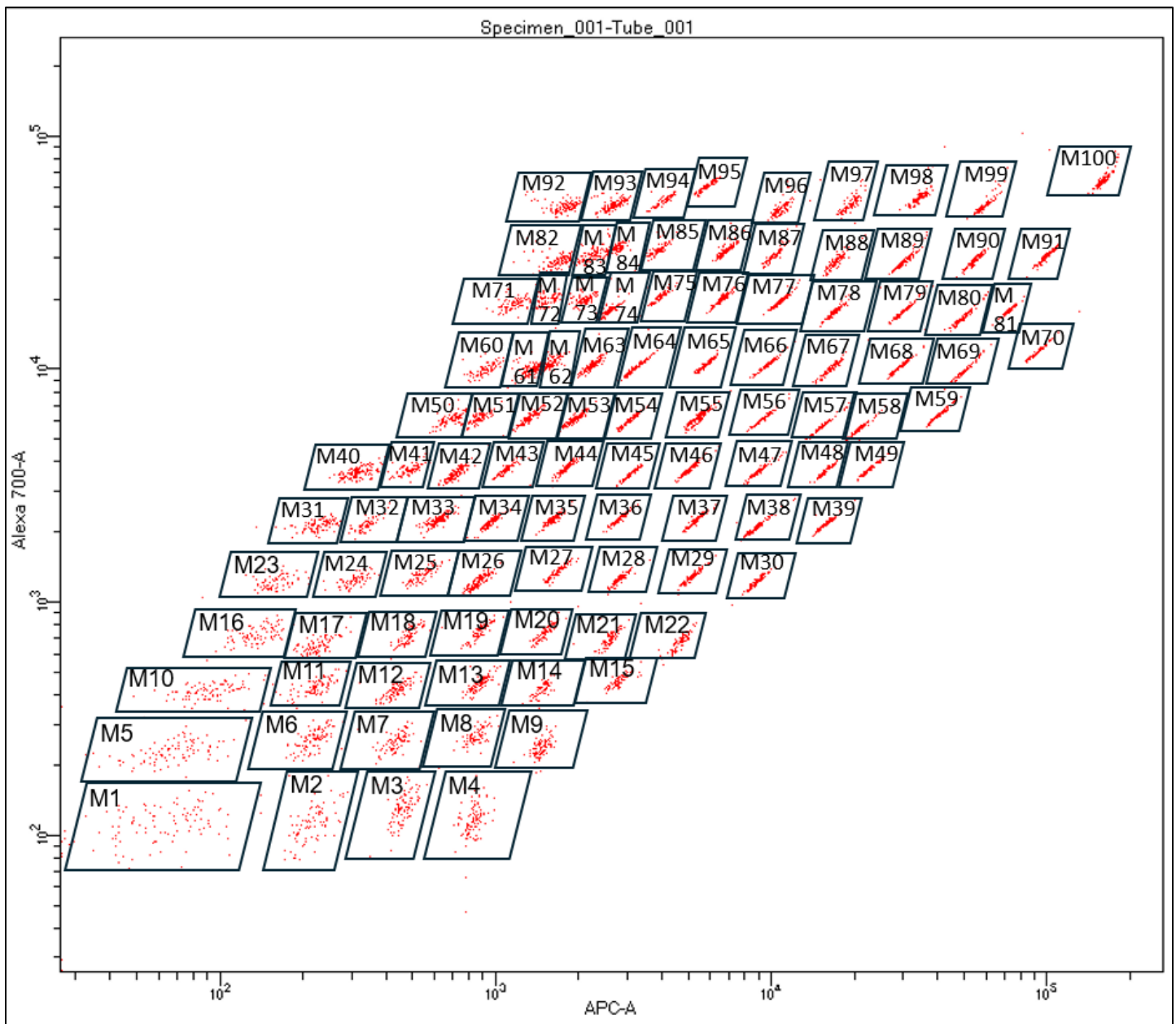


Figure 2. Different populations of beads with dual fluorescence combinations (“M#”) enable multiplex protein detection with RayPlex® arrays.

B. 96 Well Plate Layout for Different Size Kits

8-Sample Kit

Plate #1

Grp#1 Standard		Grp#1 Samples		Grp#2 Standard		Grp#2 Samples		Grp#3 Standard		Grp#3 Samples	
Std1	Std1	Sample#1	Sample#1	Std1	Std1	Sample#1	Sample#1	Std1	Std1	Sample#1	Sample#1
Std2	Std2	Sample#2	Sample#2	Std2	Std2	Sample#2	Sample#2	Std2	Std2	Sample#2	Sample#2
Std3	Std3	Sample#3	Sample#3	Std3	Std3	Sample#3	Sample#3	Std3	Std3	Sample#3	Sample#3
Std4	Std4	Sample#4	Sample#4	Std4	Std4	Sample#4	Sample#4	Std4	Std4	Sample#4	Sample#4
Std5	Std5	Sample#5	Sample#5	Std5	Std5	Sample#5	Sample#5	Std5	Std5	Sample#5	Sample#5
Std6	Std6	Sample#6	Sample#6	Std6	Std6	Sample#6	Sample#6	Std6	Std6	Sample#6	Sample#6
Std7	Std7	Sample#7	Sample#7	Std7	Std7	Sample#7	Sample#7	Std7	Std7	Sample#7	Sample#7
Neg	Neg	Sample#8	Sample#8	Neg	Neg	Sample#8	Sample#8	Neg	Neg	Sample#8	Sample#8

Plate #2

Grp#4 Standard		Grp#4 Samples		Grp#5 Standard		Grp#5 Samples		Grp#6 Standard		Grp#6 Samples	
Std1	Std1	Sample#1	Sample#1	Std1	Std1	Sample#1	Sample#1	Std1	Std1	Sample#1	Sample#1
Std2	Std2	Sample#2	Sample#2	Std2	Std2	Sample#2	Sample#2	Std2	Std2	Sample#2	Sample#2
Std3	Std3	Sample#3	Sample#3	Std3	Std3	Sample#3	Sample#3	Std3	Std3	Sample#3	Sample#3
Std4	Std4	Sample#4	Sample#4	Std4	Std4	Sample#4	Sample#4	Std4	Std4	Sample#4	Sample#4
Std5	Std5	Sample#5	Sample#5	Std5	Std5	Sample#5	Sample#5	Std5	Std5	Sample#5	Sample#5
Std6	Std6	Sample#6	Sample#6	Std6	Std6	Sample#6	Sample#6	Std6	Std6	Sample#6	Sample#6
Std7	Std7	Sample#7	Sample#7	Std7	Std7	Sample#7	Sample#7	Std7	Std7	Sample#7	Sample#7
Neg	Neg	Sample#8	Sample#8	Neg	Neg	Sample#8	Sample#8	Neg	Neg	Sample#8	Sample#8

16-Sample Kit

Plate #1

Grp#1 Standard		Grp#1 Samples				Grp#2 Standard		Grp#2 Samples			
Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9	Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9
Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10	Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10
Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11	Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11
Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12	Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12
Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13	Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13
Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14	Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14
Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15	Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15
Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16	Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16

Plate #2

Grp#3 Standard		Grp#3 Samples				Grp#4 Standard		Grp#4 Samples			
Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9	Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9
Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10	Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10
Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11	Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11
Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12	Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12
Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13	Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13
Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14	Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14
Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15	Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15
Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16	Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16

Plate #3

Grp#5 Standard		Grp#5 Samples				Grp#6 Standard		Grp#6 Samples			
Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9	Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9
Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10	Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10
Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11	Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11
Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12	Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12
Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13	Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13
Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14	Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14
Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15	Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15
Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16	Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16

40-Sample Kit**Plate #1**

Grp#1 Standard		Grp#1 Samples									
Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9	Sample#17	Sample#17	Sample#25	Sample#25	Sample#33	Sample#33
Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10	Sample#18	Sample#18	Sample#26	Sample#26	Sample#34	Sample#34
Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11	Sample#19	Sample#19	Sample#27	Sample#27	Sample#35	Sample#35
Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12	Sample#20	Sample#20	Sample#28	Sample#28	Sample#36	Sample#36
Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13	Sample#21	Sample#21	Sample#29	Sample#29	Sample#37	Sample#37
Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14	Sample#22	Sample#22	Sample#30	Sample#30	Sample#38	Sample#38
Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15	Sample#23	Sample#23	Sample#31	Sample#31	Sample#39	Sample#39
Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16	Sample#24	Sample#24	Sample#32	Sample#32	Sample#40	Sample#40

Plate #2

Grp#2 Standard		Grp#2 Samples									
Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9	Sample#17	Sample#17	Sample#25	Sample#25	Sample#33	Sample#33
Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10	Sample#18	Sample#18	Sample#26	Sample#26	Sample#34	Sample#34
Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11	Sample#19	Sample#19	Sample#27	Sample#27	Sample#35	Sample#35
Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12	Sample#20	Sample#20	Sample#28	Sample#28	Sample#36	Sample#36
Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13	Sample#21	Sample#21	Sample#29	Sample#29	Sample#37	Sample#37
Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14	Sample#22	Sample#22	Sample#30	Sample#30	Sample#38	Sample#38
Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15	Sample#23	Sample#23	Sample#31	Sample#31	Sample#39	Sample#39
Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16	Sample#24	Sample#24	Sample#32	Sample#32	Sample#40	Sample#40

Plate #3

Grp#3 Standard		Grp#3 Samples									
Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9	Sample#17	Sample#17	Sample#25	Sample#25	Sample#33	Sample#33
Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10	Sample#18	Sample#18	Sample#26	Sample#26	Sample#34	Sample#34
Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11	Sample#19	Sample#19	Sample#27	Sample#27	Sample#35	Sample#35
Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12	Sample#20	Sample#20	Sample#28	Sample#28	Sample#36	Sample#36
Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13	Sample#21	Sample#21	Sample#29	Sample#29	Sample#37	Sample#37
Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14	Sample#22	Sample#22	Sample#30	Sample#30	Sample#38	Sample#38
Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15	Sample#23	Sample#23	Sample#31	Sample#31	Sample#39	Sample#39
Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16	Sample#24	Sample#24	Sample#32	Sample#32	Sample#40	Sample#40

Plate #4

Grp#4 Standard		Grp#4 Samples									
Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9	Sample#17	Sample#17	Sample#25	Sample#25	Sample#33	Sample#33
Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10	Sample#18	Sample#18	Sample#26	Sample#26	Sample#34	Sample#34
Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11	Sample#19	Sample#19	Sample#27	Sample#27	Sample#35	Sample#35
Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12	Sample#20	Sample#20	Sample#28	Sample#28	Sample#36	Sample#36
Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13	Sample#21	Sample#21	Sample#29	Sample#29	Sample#37	Sample#37
Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14	Sample#22	Sample#22	Sample#30	Sample#30	Sample#38	Sample#38
Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15	Sample#23	Sample#23	Sample#31	Sample#31	Sample#39	Sample#39
Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16	Sample#24	Sample#24	Sample#32	Sample#32	Sample#40	Sample#40

Plate #5

Grp#5 Standard		Grp#5 Samples									
Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9	Sample#17	Sample#17	Sample#25	Sample#25	Sample#33	Sample#33
Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10	Sample#18	Sample#18	Sample#26	Sample#26	Sample#34	Sample#34
Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11	Sample#19	Sample#19	Sample#27	Sample#27	Sample#35	Sample#35
Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12	Sample#20	Sample#20	Sample#28	Sample#28	Sample#36	Sample#36
Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13	Sample#21	Sample#21	Sample#29	Sample#29	Sample#37	Sample#37
Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14	Sample#22	Sample#22	Sample#30	Sample#30	Sample#38	Sample#38
Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15	Sample#23	Sample#23	Sample#31	Sample#31	Sample#39	Sample#39
Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16	Sample#24	Sample#24	Sample#32	Sample#32	Sample#40	Sample#40

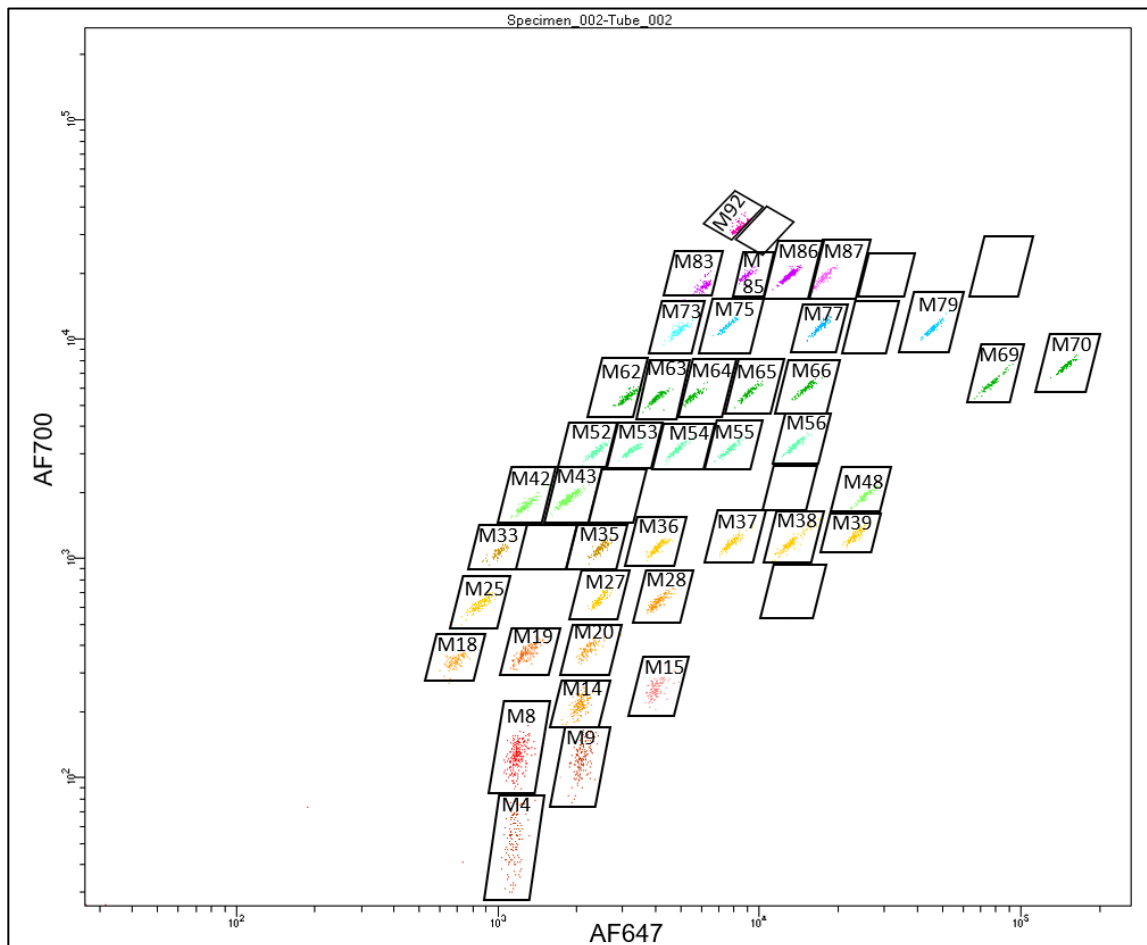
Plate #6

Grp#6 Standard		Grp#6 Samples									
Std1	Std1	Sample#1	Sample#1	Sample#9	Sample#9	Sample#17	Sample#17	Sample#25	Sample#25	Sample#33	Sample#33
Std2	Std2	Sample#2	Sample#2	Sample#10	Sample#10	Sample#18	Sample#18	Sample#26	Sample#26	Sample#34	Sample#34
Std3	Std3	Sample#3	Sample#3	Sample#11	Sample#11	Sample#19	Sample#19	Sample#27	Sample#27	Sample#35	Sample#35
Std4	Std4	Sample#4	Sample#4	Sample#12	Sample#12	Sample#20	Sample#20	Sample#28	Sample#28	Sample#36	Sample#36
Std5	Std5	Sample#5	Sample#5	Sample#13	Sample#13	Sample#21	Sample#21	Sample#29	Sample#29	Sample#37	Sample#37
Std6	Std6	Sample#6	Sample#6	Sample#14	Sample#14	Sample#22	Sample#22	Sample#30	Sample#30	Sample#38	Sample#38
Std7	Std7	Sample#7	Sample#7	Sample#15	Sample#15	Sample#23	Sample#23	Sample#31	Sample#31	Sample#39	Sample#39
Neg	Neg	Sample#8	Sample#8	Sample#16	Sample#16	Sample#24	Sample#24	Sample#32	Sample#32	Sample#40	Sample#40

C. Target Proteins and Associated Bead IDs in This Kit

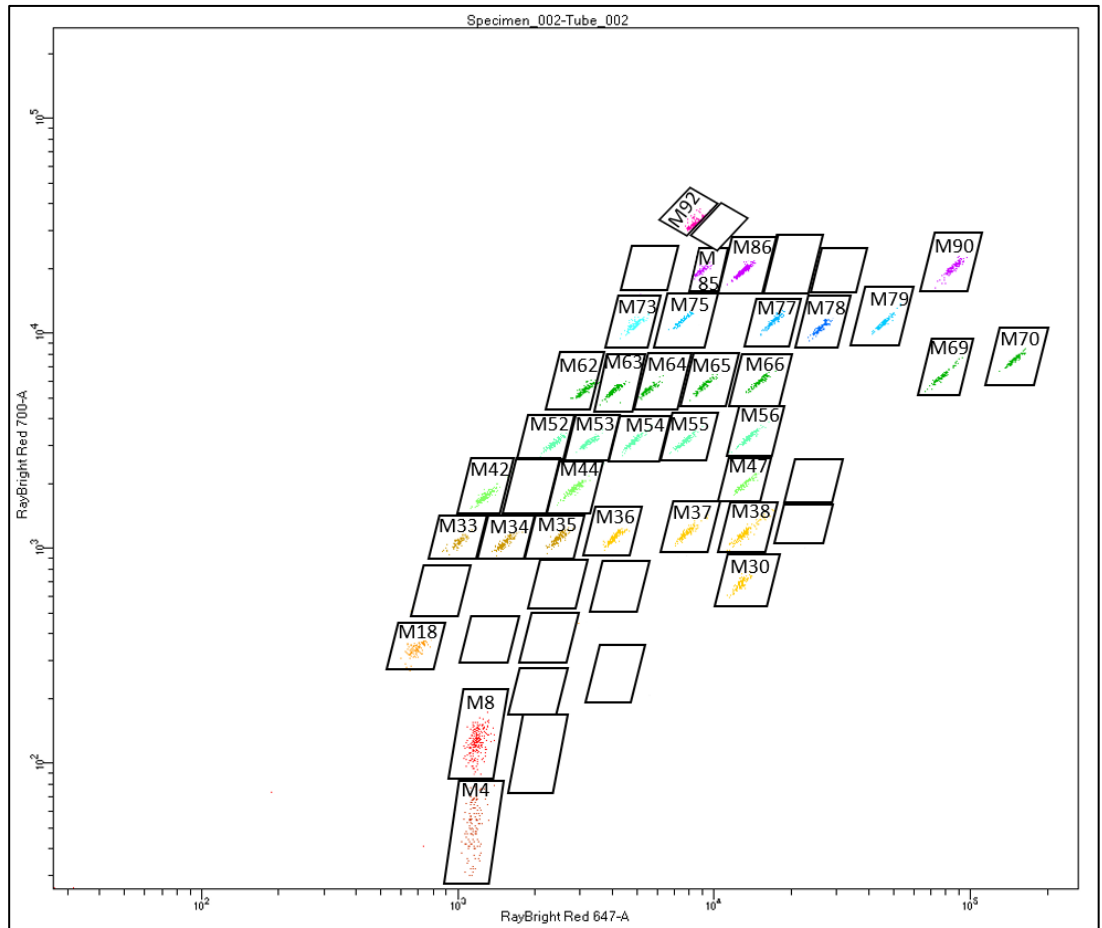
Group #1: Inflammatory Cytokines

Bead ID	Target name
M4	Eotaxin-2
M8	BLC
M9	RANTES
M14	MCP-1
M15	IL-10
M18	PDGF-BB
M19	IFN- γ
M20	TNF- α
M25	IL-5
M27	IL-1 β
M28	IL-6
M33	IL-2
M35	IL-4
M36	G-CSF
M37	IL-12 p70
M38	IL-8
M39	MIP-1 α
M42	IL-7
M43	IL-9
M48	TIMP-2
M52	TNFR1
M53	IL-1Ra
M54	TNFR2
M55	IL-17
M56	I-309
M62	IL-12 p40
M63	IL-13
M64	IL-15
M65	Eotaxin-1
M66	IL-16
M69	MIP-1 δ
M70	TNF β
M73	IL-1 α
M75	ICAM-1
M77	IL-11
M79	IFN α
M83	M-CSF
M85	GM-CSF
M86	IL-28A
M87	MIP-3 β
M92	MIG



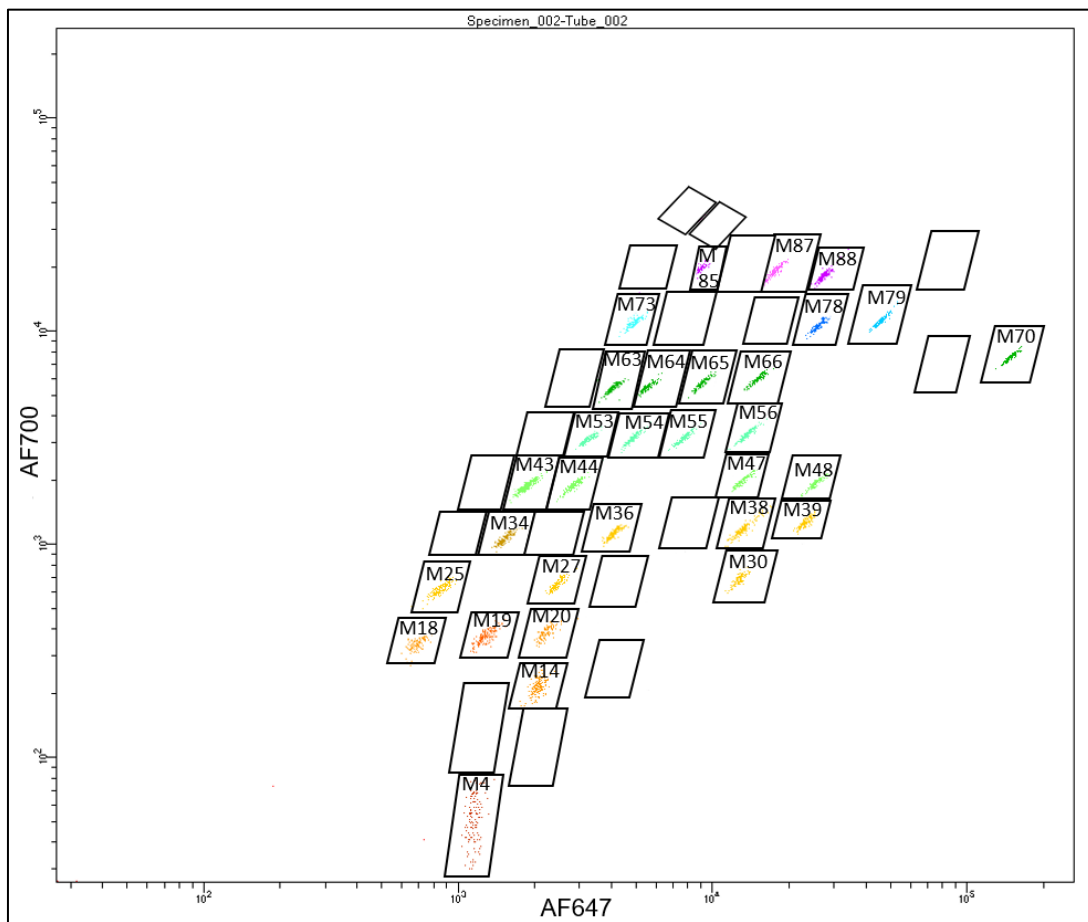
Group #2: Chemokines

Bead ID	Target name
M4	TSLP
M8	Eotaxin-3
M18	MIP-1 β
M30	MDC
M33	I-TAC
M34	TECK
M35	HCC-4
M37	IL-18 Bpa
M38	NAP-2
M42	Osteopontin
M44	MIF
M47	LIF
M52	Axl
M53	Betacellulin
M54	CXCL5
M55	Lymphotactin
M56	SDF-1 α
M62	MPIF-1
M63	GRO α
M64	PARC
M65	IP-10
M66	MIP-3 α
M69	TARC
M70	MCP-2
M73	MCP-3
M75	GCP-2
M77	HCC-1
M78	IL-31
M79	IL-29
M85	IL-17F
M86	MCP-4
M90	CCL28
M92	CTACK



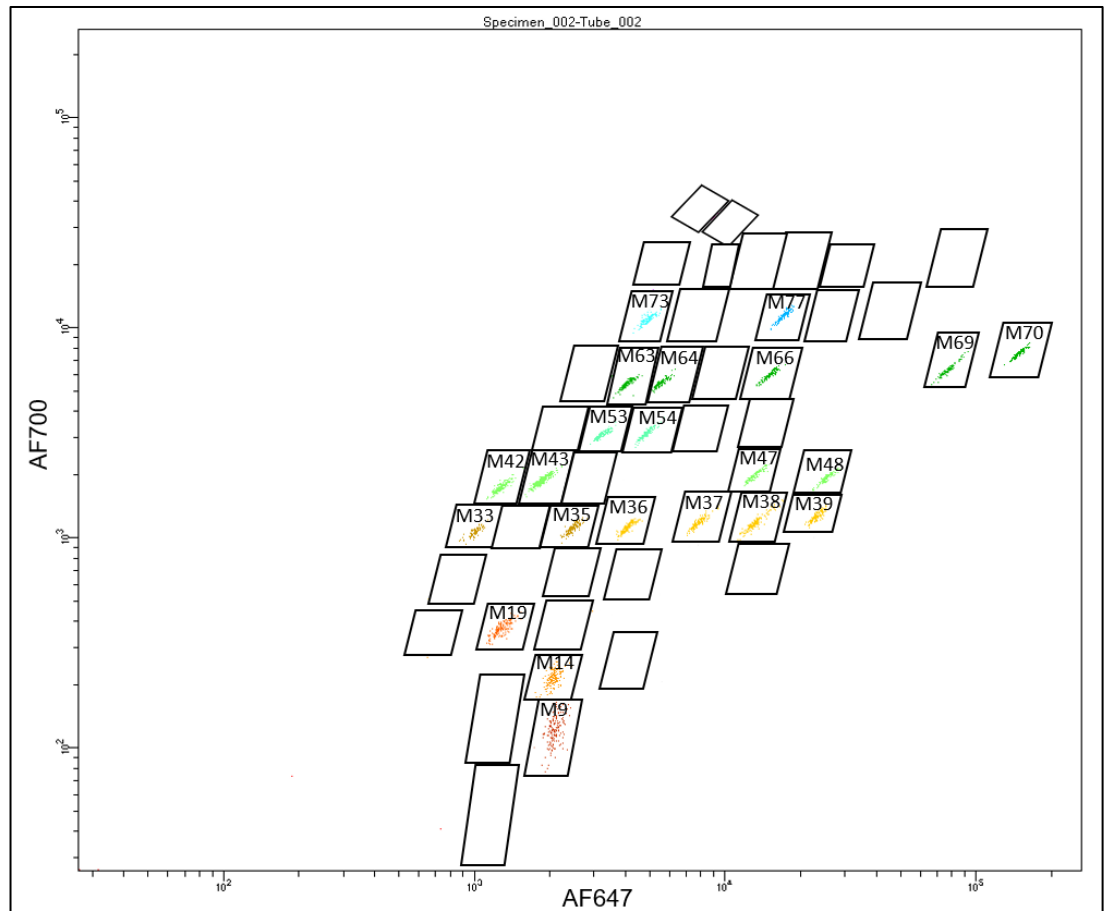
Group #3: Cytokines 1

Bead ID	Target
M4	AgRP
M14	Angiostatin
M18	LAP
M19	IL-2 R α
M20	Angiogenin
M25	PDGF-AB
M27	E-Cadherin
M30	Activin A
M33	Cripto-1
M34	ICAM-2
M36	IL-2 R β
M38	VEGFR1
M39	Follistatin
M43	Cathepsin S
M44	CD40
M47	Fc γ RIIB/C
M48	GP130
M53	Thrombopoietin
M54	DAN
M55	Galectin-7
M56	Fas Ligand
M63	SDF-1 β
M64	VEGF-C
M65	TGF β 2
M66	IL-13 R α 1
M70	IL-1 R4
M73	Tie-2
M78	TSH
M79	SHH-N
M85	TREM-1
M87	IL-23
M88	TRAIL R4



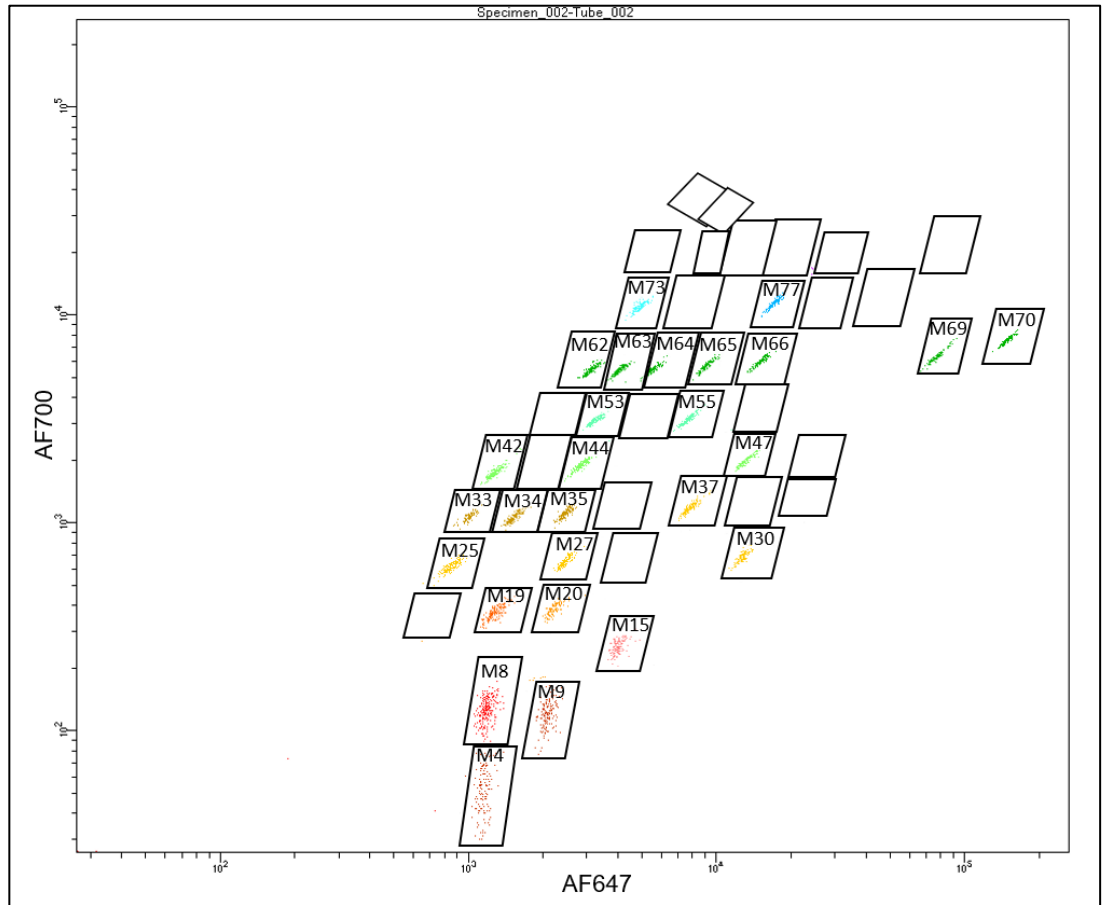
Group #4: Cytokines 2

Bead ID	Target
M9	AFP
M14	ANGPTL4
M19	CA125
M33	FSH
M35	hCG β
M36	IGF-1R
M37	IL-1 RII
M38	IL-18 R β
M39	IL-21
M42	IL-3
M43	Leptin
M47	MMP-10
M48	MMP-13
M53	MMP-3
M54	MMP-8
M63	NSE
M64	OSM
M66	Prolactin
M69	PSA-Free
M70	Siglec-9
M73	TACE
M77	TIMP-4



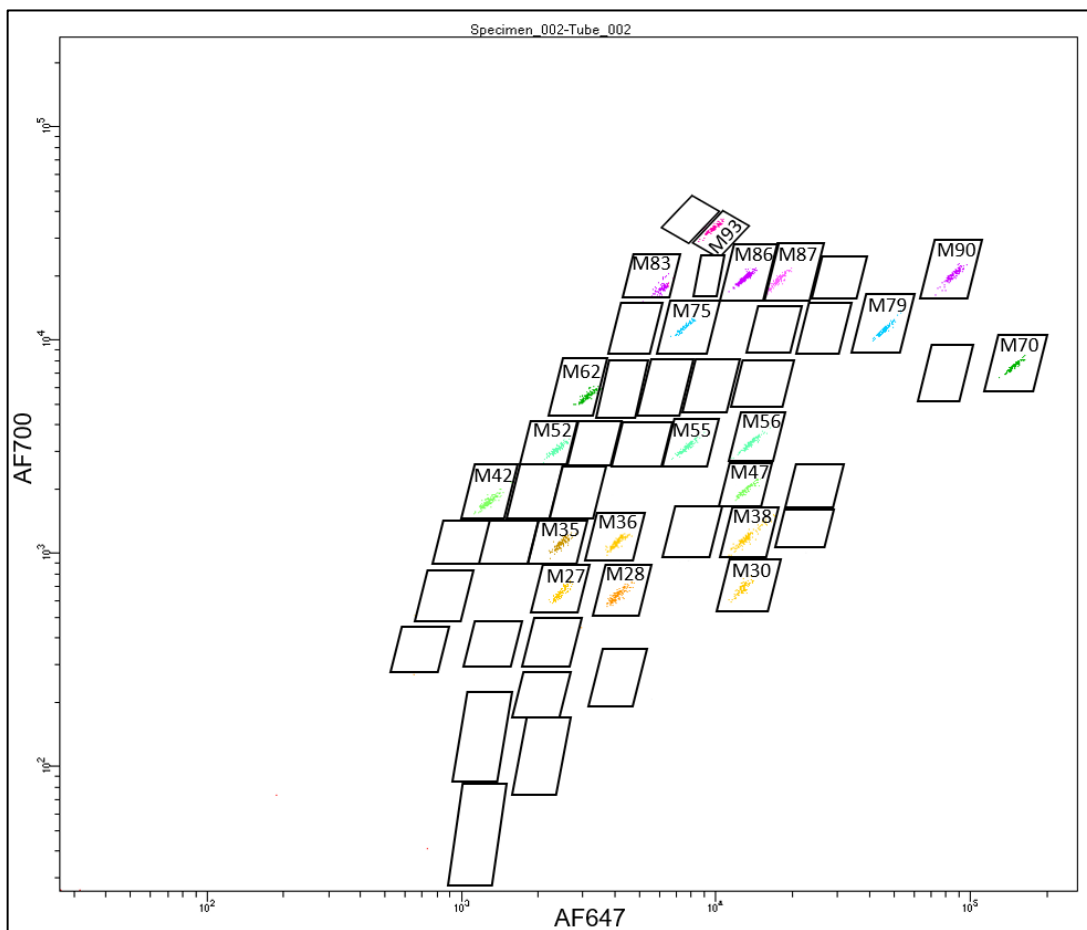
Group #5: Receptors

Bead ID	Target
M4	TRAIL R3
M8	DR6
M9	CD14
M15	CEACAM-1
M19	IL-1 R1
M20	PECAM-1
M25	E-Selectin
M27	CD105
M30	Trappin-2
M33	ErbB3
M34	CD30
M35	IL-2 R γ
M37	CD80
M42	Contactin-2
M44	uPAR
M47	Dtk
M53	ALCAM
M55	LIMP2
M62	MICA
M63	TIM-1
M64	IL-17 RA
M65	XEDAR
M66	Fas
M69	IL-21 R
M70	CD40 ligand
M73	Flt-3 ligand
M77	GITR



Group #6: Growth Factors

Bead ID	Target
M27	GDF-15
M28	OPG
M30	VEGF-D
M35	β -NGF
M36	Growth Hormone
M38	IGF-1
M42	NGFR
M47	TGF- β 3
M52	BMP-4
M55	IGFBP-2
M56	BMP-7
M62	VEGF-A
M70	bFGF
M75	TGF β 1
M79	SCF
M83	IGFBP-6
M86	VEGFR3
M87	VEGFR2
M90	PIGF
M93	EG-VEGF



V. Materials Provided

Upon receipt, the Lyophilized Protein Standard Mixes (Items 11-16) should be stored at -20°C or below. Other components should be stored at 4°C. Use reagents immediately after preparation. Additional vials of the Lyophilized Protein Standard Mixes (Items 11-16) can be purchased separately (Catalog # FAHM-CYT-175-Item11-16). If stored at the proper temperature, the kit will retain complete activity for at least 6 months.

Catalog #	Description	8-Sample Kit	16-Sample Kit	40-Sample Kit
FAHM-CYT-175-Item1	RayPlex® Magnetic Multiplex Bead Cocktail (Group #1)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAHM-CYT-175-Item2	RayPlex® Magnetic Multiplex Bead Cocktail (Group #2)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAHM-CYT-175-Item3	RayPlex® Magnetic Multiplex Bead Cocktail (Group #3)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAHM-CYT-175-Item4	RayPlex® Magnetic Multiplex Bead Cocktail (Group #4)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAHM-CYT-175-Item5	RayPlex® Magnetic Multiplex Bead Cocktail (Group #5)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAHM-CYT-175-Item6	RayPlex® Magnetic Multiplex Bead Cocktail (Group #6)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAX-SDB-Item7	20X PBS	10 mL x 1 vial	10 mL x 2 vials	10 mL x 3 vials
FAX-AD-1-Item8	1X Assay Diluent	25 mL x 2 vials	25 mL x 3 vials	25 mL x 6 vials
FAX-WB-Item9	20X Wash Buffer	20 mL x 1 vial	20 mL x 2 vials	20 mL x 3 vials
137-00022-Item10	RayBio® Human Serum/Plasma Diluent	4 mL x 2 vial	4 mL x 2 vials	4 mL x 2 vials
FAHM-CYT-175-Item11	Lyophilized Protein Standard Mix (Group #1)	2	2	2
FAHM-CYT-175-Item12	Lyophilized Protein Standard Mix (Group #2)	2	2	2
FAHM-CYT-175-Item13	Lyophilized Protein Standard Mix (Group #3)	2	2	2
FAHM-CYT-175-Item14	Lyophilized Protein Standard Mix (Group #4)	2	2	2

FAHM-CYT-175-Item15	Lyophilized Protein Standard Mix (Group #5)	2	2	2
FAHM-CYT-175-Item16	Lyophilized Protein Standard Mix (Group #6)	2	2	2
FAHM-CYT-175-Item17	Biotinylated Detection Antibody Cocktail (Group #1)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAHM-CYT-175-Item18	Biotinylated Detection Antibody Cocktail (Group #2)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAHM-CYT-175-Item19	Biotinylated Detection Antibody Cocktail (Group #3)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAHM-CYT-175-Item20	Biotinylated Detection Antibody Cocktail (Group #4)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAHM-CYT-175-Item21	Biotinylated Detection Antibody Cocktail (Group #5)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAHM-CYT-175-Item22	Biotinylated Detection Antibody Cocktail (Group #6)	0.90 mL x 1 vial	1.25 mL x 1 vial	1.25 mL x 2 vials
FAX-PE-Item23	10X PE Conjugate	500 µL x 2 vials	500 µL x 3 vials	500 µL x 6 vials
FAHM-CYT-175-Item24	Flow Cytometer Setup Bead Cocktail**	150 µL x 1 vial	150 µL x 1 vial	150 µL x 1 vial
FAX-FB-Item25	Flat-bottom 96-well Microplate	2	3	6
FAX-VB-Item26	V-Bottom 96-well Microplate	2	3	6

Notes:

1. **Flow Cytometer Setup Bead Cocktail (Item 24) is an additional aliquot of all beads used in this kit that is provided to assist in the setup of the flow cytometer. It can also be used to test for bead loss during washing prior to performing the assay. A detailed procedure for bead loss prevention is outlined in Section VI.F.
2. RayBio® Human Serum/Plasma Diluent (Item 10) is a solution that contains similar concentrations of proteins and other components shared by human serum and plasma to mimic the matrix. The diluent included in this kit is a 1:1 PBS-diluted version of the original product (Catalog #137-00022). Larger lots of the original product can be purchased here: <https://www.raybiotech.com/human-serum-plasma-diluent-137-00022>
3. RayBiotech recommends using a **Flat-Bottom 96-well Microplate (Item 25)** in conjunction with an **automated plate washing system** that uses magnetic separation.

Additional Materials Required

- Orbital 96-well plate shaker (with ability to reach 1000 rpm)
- Flow Cytometer with blue (or green) and red lasers capable of measuring phycoerythrin (PE), Alexa Fluor 700 (AF700), and Alexa Fluor 647 (AF647)/APC channels OR Luminex instrument
- Aluminum foil or 96-well Plate Aluminum Sealers
- Deionized (DI) water
- PBS
- 1.5 mL polypropylene microcentrifuge tubes or similar
- Microcentrifuge
- **Recommended**: Automated plate washing system with magnetic separation
- Optional: 96-well round bottom plate (to prepare samples)
- Optional: High Throughput Sampler (HTS) for 96-well plate reading
- Optional: Vacuum manifold compatible with a 96-well microplate if using a Filter 96-Well Microplate

VI. Assay Protocol

A. Preparation of Samples

- We recommend the following parameters for your samples: 25 µL of diluted serum, plasma, cell culture media or other fluids. Actual sample dilutions may need to be empirically determined.
- It's recommended to use a 96-well round bottom plate to prepare samples before transferring the samples to the test plate or test tubes.
- We recommend analyzing samples at least in duplicate.
- **Serum or Plasma Samples**
 - Dilute the 20X PBS (Item 7) 20-fold with deionized (DI) H₂O to prepare 1X PBS.
Example: A 20-fold dilution would be 1-part 20X PBS and 19 parts DI H₂O
 - **Serum/Plasma samples: Dilute samples 2-fold with PBS** (*diluted* Item 7) (e.g., dilute 30 µL of serum/plasma with 30 µL of PBS), **which is equal to 4-fold total dilution after mixed with beads.**
 - If further dilution is needed, RayBio® Human Serum/Plasma Diluent (Item 10) should be used.
- **Cell Culture Supernatant**
 - **Samples: No extra dilution needed, total dilution will be equal to 2-fold after mixed with beads.**
 - The use of serum (e.g., fetal bovine serum; FBS) in cell culture media can result in high background. To learn how to prepare samples, see our **Tips on Sample Preparation here:** <https://www.raybiotech.com/tips-on-sample-preparation/>
 - If cell culture supernatant from serum-containing media is tested, it is highly recommended that complete medium be used as diluent to reconstitute the protein standard since many types of serum contain proteins that may cross-react with the antibodies.
 - For serum-free media, 1X Assay Diluent (Item 8) should be used to dilute samples.
- A preliminary assay before testing your precious samples is recommended.

Note: Levels of target protein(s) may vary between different samples. Optimal dilution

factors for each sample must be determined empirically by the investigator.

Note: *Matrix effects are a common cause of non-linear dilution responses. This can occur when proteins or other components within the sample affect the immunoreactivity of the target molecule. These matrix components can also affect the ability of the antibody to recognize its target within the sample. Auto-antibodies, binding proteins, hemolysis, or certain disease states can contribute to this phenomenon. If matrix effects are suspected, centrifuge the sample(s), and dilute further such that matrix effects become negligible.*

Note: *If you experience high background or the readings exceed the detection range, further dilution of your sample is recommended.*

Note: *If you are using an automatic plate washing system that is not the same as defined in this manual, it is recommended to perform a bead loss prevention check as outlined in Section VI.F.*

B. Preparation of Reagents

- Keep all reagents on ice
 - Protect fluorescent multiplex beads from light
1. Dilute and prepare only what is needed to perform the tests for each experiment.
 2. Vortex RayPlex® Magnetic Multiplex Bead Cocktail (Items 1-6) for 30 seconds before use. Use 25 µL beads per test.
 3. Dilute the 20X Wash Buffer (Item 9) 20-fold with DI H₂O to prepare 1X Wash Buffer (see example note below).

Example: A 20-fold dilution would be 1-part 20X Wash Buffer and 19 parts DI H₂O

4. Dilute the 10X PE Conjugate (Item 23) 10-fold in 1X Assay Diluent (Item 8) to prepare a 1X PE Conjugate working stock. Use 50 µL per test.

C. Preparation of Protein Standards

NOTE: *Use the Lyophilized Protein Standard Mix (Items 11-16) within 1 hour of preparation.*

NOTE: *When testing serum or plasma, RayBio® Human Serum/Plasma Diluent (Item 10) should be used to reconstitute and serially dilute the standard mixture.*

1. Centrifuge the Lyophilized Protein Standard Mix (Items 11-16) briefly (1-3 seconds) to collect contents to the bottom of the vial.

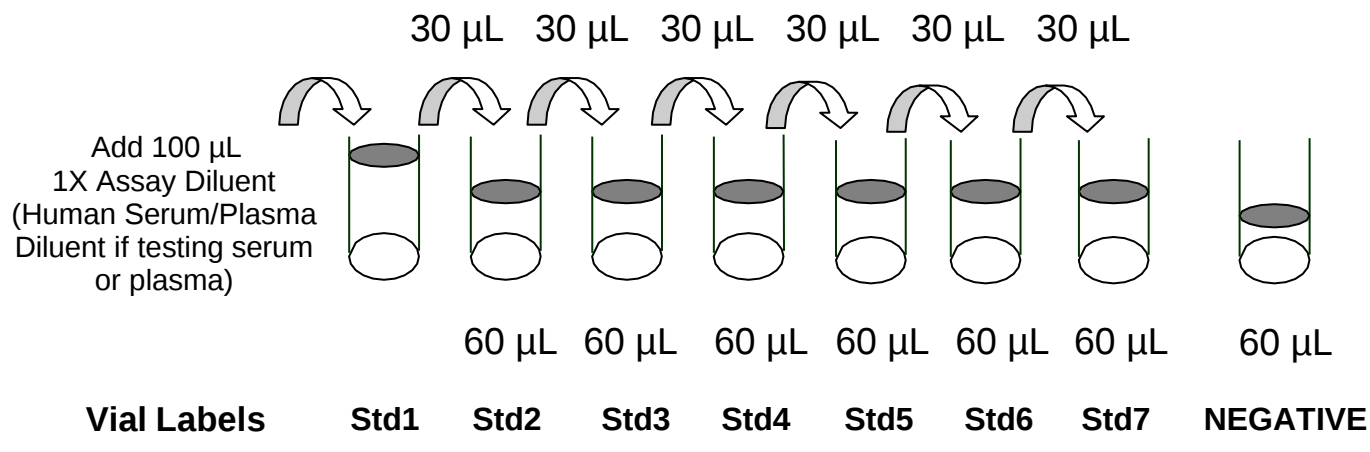
2. Reconstitute the Lyophilized Protein Standard Mix (Items 11-16):

- For **serum or plasma samples**, add 100 μ L RayBio® Human Serum/Plasma Diluent (Item 10) to the tube. Dissolve the powder thoroughly and gently by pipetting up and down 5-10 times. Label this tube as Std1. Further dilution of the standards should use the same RayBio® Human Serum/Plasma Diluent.
- For **serum-free media or non-serum fluids** add 100 μ L 1X Assay Diluent (Item 8) or serum-free media to the tube.
- For **media containing bovine serum**, such as cell culture media, complete media should be used to reconstitute the protein standard with the same procedures as above.

3. Obtain six clean 1.5 mL microcentrifuge tubes (or use a 96-well round-bottom plate). Label the tubes/wells as Std2 to Std7. Add 60 μ L 1X Assay Diluent (Item 8) or RayBio® Human Serum/Plasma Diluent (Item 10) as applicable to each tube.

4. Pipette 30 μ L Std1 into tube Std2 and mix gently. Perform 5 more serial dilutions by adding 30 μ L of Std2 to tube Std3, mix, and so on. (See also image below).

Prepare Serial Dilutions of Protein Standards



5. Add 60 μ L 1X Assay Diluent (Item 8) or RayBio® Human Serum/Plasma Diluent (Item 10) to another tube labeled as NEGATIVE. Do not add standard or samples to the NEGATIVE tube. This tube will be used as the negative control.

NOTE: The exact concentrations of each standard protein for the Std1 to Std7 serial dilutions in this kit can be found in Section VII.

D. Assay Procedure

1. Prepare a Flat-Bottom 96-well plate (Item 25), V-bottom plate (Item 26) or 1.2 µm filter plate (not supplied) and mark positions for the standards and samples. Duplicate tests are recommended for all standards and samples.

NOTE: We recommend using a Flat-Bottom 96-well plate (Item 25) in conjunction with a magnetic plate washing system. Data in this manual were obtained using a **LifeSep™ magnetic separator unit** and **BioTek 405 TS Washer**. Please note that if alternative systems are used, unforeseen bead loss may occur. It is highly recommended for investigators to follow the procedure outlined in Section VI.F to check for bead loss prior to beginning the assay.

NOTE: A Filter 96-well plate can be used for the washing steps if desired. The filter plate requires a vacuum manifold compatible with a standard 96-well microplate. Alternatively, a V-Bottom 96-well microplate can be used along with a centrifuge.

2. Add 25 µL of RayPlex® Multiplex Bead Cocktail (Items 1-6) to each well that will contain the NEGATIVE, Standard, or Sample.
3. Add 25 µL of the NEGATIVE, Standard, or Sample to the appropriate well. The total volume in each well is now 50 µL (**serum or plasma sample is diluted 4-fold totally with beads in 50 µL reaction system, cell culture supernatant / serum-free media is diluted 2-fold totally with beads**). Place plate on an orbital plate shaker. Shake at 1000 rpm at room temperature for 2 hours.

NOTE: Data collected for this kit used the above procedure for incubation; however, the incubation step can also be performed overnight at 4°C, which may increase the signal-to-noise ratio, particularly for proteins with low concentrations. The optimal incubation length (i.e. room temperature for 2 hours or overnight at 4°C) for the experiment must be determined empirically by the investigator.

4. Wash the beads using 1X Wash Buffer (*diluted* Item 9; See Section B).
 - **Automated Washing System:** Place the **Flat-Bottom 96-well Microplate (Item 25)** on the magnetic separator unit for 5 minutes to allow the magnetic beads to migrate to the bottom. Place the Flat-Bottom 96-well Microplate and Magnetic Separator Unit on the platform of an automated plate washer and perform one

wash cycle. One wash cycle should include two rounds of dispensing 200 µL of 1X Wash Buffer (*diluted* Item 9) and aspiration. It is recommended to adjust the settings such that the first aspiration leaves approximately 2 mm of 1X Wash Buffer, and the final aspiration removes all liquid. It is pivotal that all liquid is removed at the end of washing. Remove the plate from the magnetic separator unit once washing is complete. Exact washing procedures may vary depending on the automated system used.

- **V-bottom 96-well Microplate:** Add 200 µL 1X Wash Buffer (*diluted* Item 9) to each well. Spin the V-bottom Microplate (Item 26) down at 1000 g for 5 minutes at room temperature and remove the supernatant using a multichannel pipette. Repeat once more.
 - **Filter 96-well Microplate:** Add 200 µL 1X Wash Buffer (*diluted* Item 9) to each well. Turn on the vacuum, open the valve, and place the filter plate on the vacuum. Do not allow pressure to exceed 10 inches Hg (254 mm Hg). Close the valve and remove the plate when the wells have drained. Repeat once more.
5. Add 25 µL of Biotinylated Detection Antibody Cocktail (Items 17-22) to each well. Resuspend the beads by gently pipetting and incubate on an orbital shaker at 1000 rpm at room temperature for 1 hour.
 6. Wash plate as outlined in Step 4.
 7. Add 50 µL of 1X PE Conjugate (*diluted* Item 23; See Section B) to each well, incubate on an orbital shaker at 1000 rpm at room temperature for 30 minutes.
 8. Wash plate as outlined in Step 4.
 9. Resuspend in 150 µL of 1X Wash Buffer (*diluted* Item 9).
 10. Prepare samples for analysis on a flow cytometer:
 - Automated High Throughput Sampler (HTS): if using a Flat-Bottom plate, directly read samples from the plate. If using a Filter-plate, transfer samples to a V-Bottom plate (Item 26).
 - Manual reading: transfer samples to compatible tubes.
 11. Analyze samples on a flow cytometer (see Section E).

E. Flow Cytometer Set-up and Data Acquisition

NOTE: The RayPlex® Human Cytokine-175 Magnetic Bead Array Kit requires a flow cytometer that is equipped with blue (or green) and red lasers capable of detecting fluorescence in the PE, AF700, and AF647/APC channels or a Luminex instrument. The instructions below describe the set up for a flow cytometer. For Luminex instrument set up, please refer to the instrument manual.

NOTE: Perform standard QC and optimization steps to prepare the flow cytometer, then set up the flow cytometer as you would for normal use. Manual adjustment of the PE, AF700, and AF647/APC compensation may be necessary to decrease the spill-over of AF647/APC signal into PE channel.

NOTE: These instructions are general for all RayPlex® assays. The target count, and bead populations may differ from the assay above. Please see your assay's specific manual Section IV and VII for the bead populations present in your array.

1. Depending on the brand of the flow cytometer, you may need to start the acquisition software and run Quality Control beads before proceeding further.

Note: The Quality Controls beads are fluorescent particles that are used for flow cytometer setup, e.g., CS&T beads for a BD flow cytometer.

2. Start a new experiment with PE, AF700, and AF647/APC channels.
3. Resuspend 100 µL of the provided Flow Cytometer Setup Bead Cocktail (Item 24) in 200 µL 1X Wash Buffer (*diluted* Item 9) and run them on the flow cytometer.
4. Create an FSC-H/FSC-A daughter population for “Single beads” selection to remove and limit doublets or higher complexes (Figure 3A).
5. Create a new dot plot from the “Single beads” parent gate P1, adjust voltage for FSC (forward scatter, linear mode) and SSC (side scatter, linear mode) so that the bead population is clearly defined. Then create a gate around the beads (Figure 3B).
6. Create a new dot plot from the beads parent gate M. Create gates for all bead populations for the assay based on AF700 (log mode) and AF647/APC (log mode). Adjust PMT voltage of AF700 and AF647/APC channels so that all populations are well separated throughout in a prominent area (Figure 3C).

7. Create a statistical view to show the PE MFI for each population (Figure 3D-E).
8. Run a small amount of beads from the NEGATIVE control. Adjust the PE voltage so that the PE median fluorescent intensity (MFI) is around 10^{2-3} for each bead population.

Note: *To keep the testing consistent for each assay, we recommend the use of Rainbow Particles, Rainbow Beads, or a similar product to standardize the assay if they are run prior to every analysis. The optimized MFI for both the AF647/APC and PE channels for the Rainbow Particles allow the experiment to be consistent from time to time and machine to machine.*

9. Set the number of bead events to be acquired to at least 200 per target population for best results. More beads will improve data accuracy.
10. Following setup, run the standards and samples. The MFI of all analytes and samples can be exported in Excel format by “Batch Analysis” of a whole specimen in FACSDiva. Researchers can also export raw data as “FCS” files and analyze in FlowJo software.

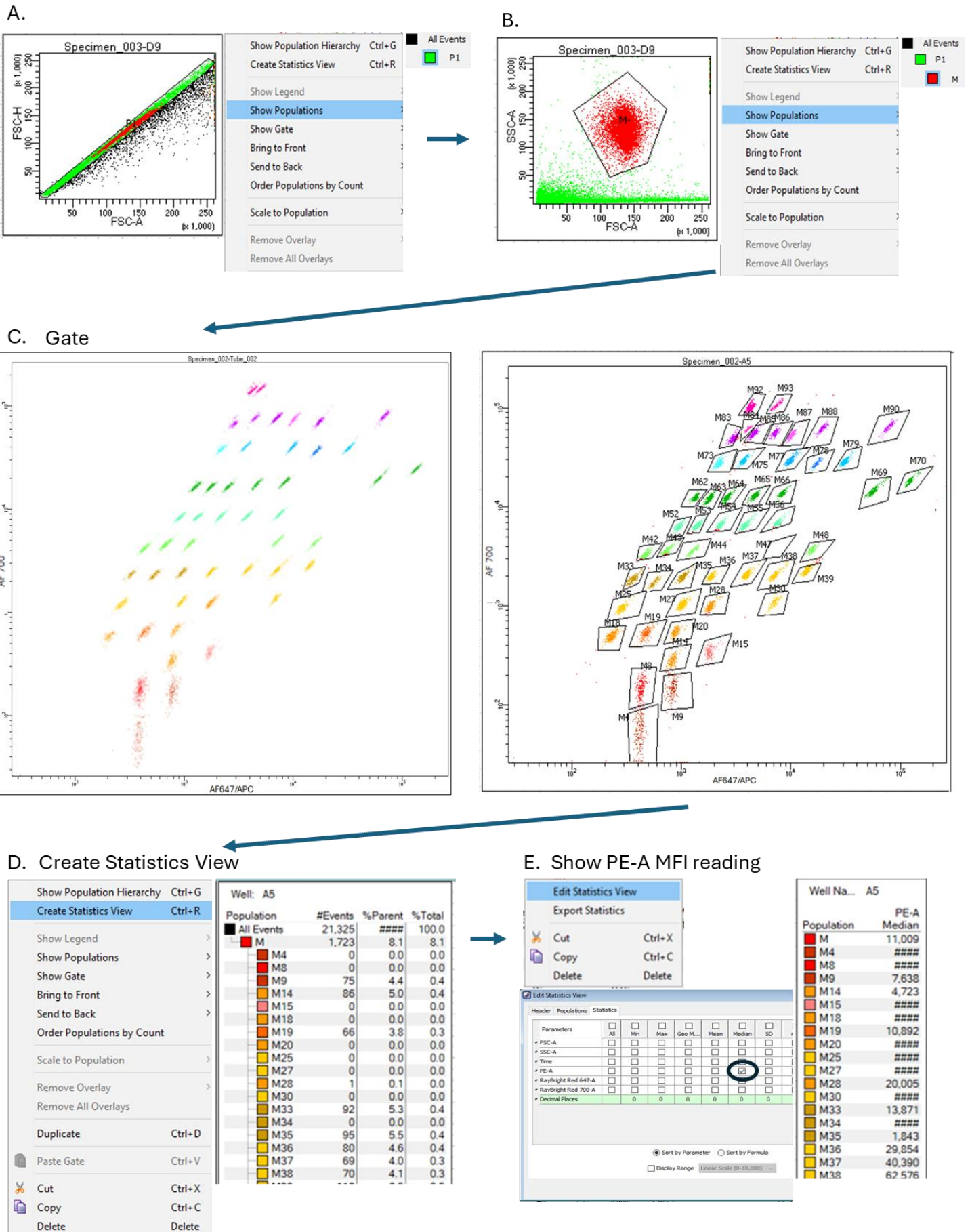


Figure 3. Representative Images of Gated Flow Cytometry Scatter Plots Using RayPlex® Arrays with M-100 series beads. (A) Single beads are used to (B) create the parent bead gate M. (C) Bead populations are separated with dual fluorescence and gated. (D, E) Statistics view is created and edited to show PE-A MFI reading. All PE-A Median values should be selected and exported based on gates of (C).

NOTE: The above figures are for demonstration purposes only.

F. Bead Loss Prevention Check

NOTE: Due to differences in automated plate washing systems, it is recommended to perform a bead loss prevention check prior to starting the assay to prevent loss of beads if an automated washing machine is being used.

1. Resuspend the contents of the **Flow Cytometer Setup Bead Cocktail (Item 24)** and add 25 µL to one well of a 96-well plate.
2. Add 25 µL of 1X Wash Buffer (*diluted* Item 9) to the same well to mimic the assay protocol.
3. Perform one wash cycle using the automated plate washing system.
 - One wash cycle should always consist of two rounds of 200 µL 1X Wash Buffer being added then removed.
4. Repeat steps 2 and 3 two more times for a total of 3 wash cycles.
5. Resuspend the beads in 150 µL of 1X Wash Buffer (*diluted* Item 9). In normal conditions, 50 µL should have more than 100 events for each bead ID.
6. Run the sample either by transferring to tubes and running in “Tube Mode” or directly from the plate with HTS mode. Optional: Run a sample of unwashed beads to compare beads with and without washing.

Note: If the bead count is less than 100 bead events for any bead IDs, it is recommended to reconsider the washing procedure used for the assay. Bead events should ideally be more than 200 for each bead ID. Using a filter-plate for washing will prevent bead loss most effectively.

G. Data Analysis

1. The MFI of all analytes and samples can be exported in Excel format by “Batch Analysis” of a whole specimen in FACSDiva (See **Appendix: RayPlex® Data Analysis Using BD FACS Diva Batch Analysis**).
2. MFI data of each analyte for all samples can also be acquired by exporting the entire dataset as FCS files. Analyze data in FlowJo or equivalent software and export the PE MFI into an Excel (or similar) format.

VII. Assay Information

A. 8-Point Standard Protein Concentrations (pg/mL)

The concentrations of target proteins corresponding to standard (Std) 1-7 serial dilutions are displayed below in pg/mL. These concentrations are used to produce the standard curves.

Group #1: Inflammatory Cytokines

Target	STD1	STD2	STD3	STD4	STD5	STD6	STD7	CTRL
Eotaxin-2	4000.0	1333.3	444.4	148.1	49.4	16.5	5.5	0.0
BLC	1500.0	500.0	166.7	55.6	18.5	6.2	2.1	0.0
RANTES	1500.0	500.0	166.7	55.6	18.5	6.2	2.1	0.0
MCP-1	2000.0	666.7	222.2	74.1	24.7	8.2	2.7	0.0
IL-10	1000.0	333.3	111.1	37.0	12.3	4.1	1.4	0.0
PDGF-BB	1500.0	500.0	166.7	55.6	18.5	6.2	2.1	0.0
IFN- γ	600.0	200.0	66.7	22.2	7.4	2.5	0.8	0.0
TNF- α	1500.0	500.0	166.7	55.6	18.5	6.2	2.1	0.0
IL-5	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
IL-1 β	6000.0	2000.0	666.7	222.2	74.1	24.7	8.2	0.0
IL-6	6000.0	2000.0	666.7	222.2	74.1	24.7	8.2	0.0
IL-2	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
IL-4	2000.0	666.7	222.2	74.1	24.7	8.2	2.7	0.0
G-CSF	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
IL-12 p70	2000.0	666.7	222.2	74.1	24.7	8.2	2.7	0.0
IL-8	4000.0	1333.3	444.4	148.1	49.4	16.5	5.5	0.0
MIP-1 α	1500.0	500.0	166.7	55.6	18.5	6.2	2.1	0.0
IL-7	1200.0	400.0	133.3	44.4	14.8	4.9	1.6	0.0
IL-9	5000.0	1666.7	555.6	185.2	61.7	20.6	6.9	0.0
TIMP-2	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
TNFR1	30000.0	10000.0	3333.3	1111.1	370.4	123.5	41.2	0.0
IL-1Ra	30000.0	10000.0	3333.3	1111.1	370.4	123.5	41.2	0.0
TNFR2	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
IL-17	5000.0	1666.7	555.6	185.2	61.7	20.6	6.9	0.0
I-309	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
IL-12 p40	20000.0	6666.7	2222.2	740.7	246.9	82.3	27.4	0.0
IL-13	1000.0	333.3	111.1	37.0	12.3	4.1	1.4	0.0
IL-15	30000.0	10000.0	3333.3	1111.1	370.4	123.5	41.2	0.0
Eotaxin-1	1500.0	500.0	166.7	55.6	18.5	6.2	2.1	0.0
IL-16	30000.0	10000.0	3333.3	1111.1	370.4	123.5	41.2	0.0
MIP-1 β	30000.0	10000.0	3333.3	1111.1	370.4	123.5	41.2	0.0
TNF β	30000.0	10000.0	3333.3	1111.1	370.4	123.5	41.2	0.0
IL-1 α	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
ICAM-1	200000.0	66666.7	22222.2	7407.4	2469.1	823.0	274.3	0.0
IL-11	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
IFN α	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
M-CSF	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
GM-CSF	30000.0	10000.0	3333.3	1111.1	370.4	123.5	41.2	0.0
IL-28A	5000.0	1666.7	555.6	185.2	61.7	20.6	6.9	0.0
MIP-3 β	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
MIG	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0

Group #2: Chemokines

Target	STD1	STD2	STD3	STD4	STD5	STD6	STD7	CTRL
TSLP	2000.0	666.7	222.2	74.1	24.7	8.2	2.7	0.0
Eotaxin-3	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
MIP-1 β	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
MDC	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
I-TAC	5000.0	1666.7	555.6	185.2	61.7	20.6	6.9	0.0
TECK	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
HCC-4	5000.0	1666.7	555.6	185.2	61.7	20.6	6.9	0.0
IL-18 Bpa	20000.0	6666.7	2222.2	740.7	246.9	82.3	27.4	0.0
NAP-2	200000.0	66666.7	22222.2	7407.4	2469.1	823.0	274.3	0.0
Osteopontin	200000.0	66666.7	22222.2	7407.4	2469.1	823.0	274.3	0.0
MIF	300.0	100.0	33.3	11.1	3.7	1.2	0.4	0.0
LIF	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
Axl	20000.0	6666.7	2222.2	740.7	246.9	82.3	27.4	0.0
Betacellulin	30000.0	10000.0	3333.3	1111.1	370.4	123.5	41.2	0.0
CXCL5	6000.0	2000.0	666.7	222.2	74.1	24.7	8.2	0.0
Lymphotactin	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
SDF-1 α	2000.0	666.7	222.2	74.1	24.7	8.2	2.7	0.0
MPIF-1	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
GRO α	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
PARC	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
IP-10	15000.0	5000.0	1666.7	555.6	185.2	61.7	20.6	0.0
MIP-3 α	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
TARC	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
MCP-2	1000.0	333.3	111.1	37.0	12.3	4.1	1.4	0.0
MCP-3	500.0	166.7	55.6	18.5	6.2	2.1	0.7	0.0
GCP-2	6000.0	2000.0	666.7	222.2	74.1	24.7	8.2	0.0
HCC-1	6000.0	2000.0	666.7	222.2	74.1	24.7	8.2	0.0
IL-31	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
IL-29	50000.0	16666.7	5555.6	1851.9	617.3	205.8	68.6	0.0
IL-17F	6000.0	2000.0	666.7	222.2	74.1	24.7	8.2	0.0
MCP-4	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
CCL28	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
CTACK	20000.0	6666.7	2222.2	740.7	246.9	82.3	27.4	0.0

Group #3: Cytokines 1

Target	STD1	STD2	STD3	STD4	STD5	STD6	STD7	CTRL
AgRP	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
Angiostatin	40000.0	13333.3	4444.4	1481.5	493.8	164.6	54.9	0.0
LAP	15000.0	5000.0	1666.7	555.6	185.2	61.7	20.6	0.0
IL-2 R α	20000.0	6666.7	2222.2	740.7	246.9	82.3	27.4	0.0
Angiogenin	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
PDGF-AB	1000.0	333.3	111.1	37.0	12.3	4.1	1.4	0.0
E-Cadherin	200000.0	66666.7	22222.2	7407.4	2469.1	823.0	274.3	0.0
Activin A	80000.0	26666.7	8888.9	2963.0	987.7	329.2	109.7	0.0
Cripto-1	20000.0	6666.7	2222.2	740.7	246.9	82.3	27.4	0.0
ICAM-2	350000.0	116666.7	38888.9	12963.0	4321.0	1440.3	480.1	0.0
IL-2 R β	300000.0	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	0.0
VEGFR1	80000.0	26666.7	8888.9	2963.0	987.7	329.2	109.7	0.0
Follistatin	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
Cathepsin S	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
CD40	40000.0	13333.3	4444.4	1481.5	493.8	164.6	54.9	0.0
Fc γ RIIB/C	180000.0	60000.0	20000.0	6666.7	2222.2	740.7	246.9	0.0
GP130	400000.0	133333.3	44444.4	14814.8	4938.3	1646.1	548.7	0.0
Thrombopoietin	200000.0	66666.7	22222.2	7407.4	2469.1	823.0	274.3	0.0
DAN	40000.0	13333.3	4444.4	1481.5	493.8	164.6	54.9	0.0
Galectin-7	180000.0	60000.0	20000.0	6666.7	2222.2	740.7	246.9	0.0
Fas Ligand	1000.0	333.3	111.1	37.0	12.3	4.1	1.4	0.0
SDF-1 β	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
VEGF-C	40000.0	13333.3	4444.4	1481.5	493.8	164.6	54.9	0.0
TGF β 2	60000.0	20000.0	6666.7	2222.2	740.7	246.9	82.3	0.0
IL-13 R α 1	80000.0	26666.7	8888.9	2963.0	987.7	329.2	109.7	0.0
IL-1 R4	5000.0	1666.7	555.6	185.2	61.7	20.6	6.9	0.0
Tie-2	80000.0	26666.7	8888.9	2963.0	987.7	329.2	109.7	0.0
TSH	150000.0	50000.0	16666.7	5555.6	1851.9	617.3	205.8	0.0
SHH-N	7000.0	2333.3	777.8	259.3	86.4	28.8	9.6	0.0
TREM-1	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
IL-23	100.0	33.3	11.1	3.7	1.2	0.4	0.1	0.0
TRAIL R4	8000.0	2666.7	888.9	296.3	98.8	32.9	11.0	0.0

Group #4: Cytokines 2

Target	STD1	STD2	STD3	STD4	STD5	STD6	STD7	CTRL
AFP	150000.0	50000.0	16666.7	5555.6	1851.9	617.3	205.8	0.0
ANGPTL4	5000.0	1666.7	555.6	185.2	61.7	20.6	6.9	0.0
CA125	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
FSH	150000.0	50000.0	16666.7	5555.6	1851.9	617.3	205.8	0.0
hCG β	500.0	166.7	55.6	18.5	6.2	2.1	0.7	0.0
IGF-1R	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
IL-1 RII	50000.0	16666.7	5555.6	1851.9	617.3	205.8	68.6	0.0
IL-18 R β	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
IL-21	50000.0	16666.7	5555.6	1851.9	617.3	205.8	68.6	0.0
IL-3	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
Leptin	5000.0	1666.7	555.6	185.2	61.7	20.6	6.9	0.0
MMP-10	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
MMP-13	5000.0	1666.7	555.6	185.2	61.7	20.6	6.9	0.0
MMP-3	150000.0	50000.0	16666.7	5555.6	1851.9	617.3	205.8	0.0
MMP-8	150000.0	50000.0	16666.7	5555.6	1851.9	617.3	205.8	0.0
NSE	180000.0	60000.0	20000.0	6666.7	2222.2	740.7	246.9	0.0
OSM	20000.0	6666.7	2222.2	740.7	246.9	82.3	27.4	0.0
Prolactin	150000.0	50000.0	16666.7	5555.6	1851.9	617.3	205.8	0.0
PSA-Free	200000.0	66666.7	22222.2	7407.4	2469.1	823.0	274.3	0.0
Siglec-9	20000.0	6666.7	2222.2	740.7	246.9	82.3	27.4	0.0
TACE	200000.0	66666.7	22222.2	7407.4	2469.1	823.0	274.3	0.0
TIMP-4	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0

Group #5: Receptors

Target	STD1	STD2	STD3	STD4	STD5	STD6	STD7	CTRL
TRAIL R3	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
DR6	85000.0	28333.3	9444.4	3148.1	1049.4	349.8	116.6	0.0
CD14	90000.0	30000.0	10000.0	3333.3	1111.1	370.4	123.5	0.0
CEACAM-1	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
IL-1 R1	80000.0	26666.7	8888.9	2963.0	987.7	329.2	109.7	0.0
PECAM-1	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
E-Selectin	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
CD105	33300.0	11100.0	3700.0	1233.3	411.1	137.0	45.7	0.0
Trappin-2	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
ErbB3	80000.0	26666.7	8888.9	2963.0	987.7	329.2	109.7	0.0
CD30	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
IL-2 R γ	30000.0	10000.0	3333.3	1111.1	370.4	123.5	41.2	0.0
CD80	60000.0	20000.0	6666.7	2222.2	740.7	246.9	82.3	0.0
Contactin-2	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
uPAR	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
Dtk	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
ALCAM	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
LIMP2	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
MICA	90000.0	30000.0	10000.0	3333.3	1111.1	370.4	123.5	0.0
TIM-1	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
IL-17 RA	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
XEDAR	150000.0	50000.0	16666.7	5555.6	1851.9	617.3	205.8	0.0
Fas	50000.0	16666.7	5555.6	1851.9	617.3	205.8	68.6	0.0
IL-21 R	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
CD40 ligand	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
Flt-3 ligand	50000.0	16666.7	5555.6	1851.9	617.3	205.8	68.6	0.0
GITR	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0

Group #6: Growth Factors

Target	STD1	STD2	STD3	STD4	STD5	STD6	STD7	CTRL
GDF-15	11110.0	3703.3	1234.4	411.5	137.2	45.7	15.2	0.0
OPG	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
VEGF-D	33330.0	11110.0	3703.3	1234.4	411.5	137.2	45.7	0.0
β -NGF	10000.0	3333.3	1111.1	370.4	123.5	41.2	13.7	0.0
Growth Hormone	80000.0	26666.7	8888.9	2963.0	987.7	329.2	109.7	0.0
IGF-1	150000.0	50000.0	16666.7	5555.6	1851.9	617.3	205.8	0.0
NGFR	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
TGF- β 3	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
BMP-4	20000.0	6666.7	2222.2	740.7	246.9	82.3	27.4	0.0
IGFBP-2	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
BMP-7	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
VEGF-A	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
bFGF	3000.0	1000.0	333.3	111.1	37.0	12.3	4.1	0.0
TGF β 1	200000.0	66666.7	22222.2	7407.4	2469.1	823.0	274.3	0.0
SCF	25000.0	8333.3	2777.8	925.9	308.6	102.9	34.3	0.0
IGFBP-6	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
VEGFR3	100000.0	33333.3	11111.1	3703.7	1234.6	411.5	137.2	0.0
VEGFR2	150000.0	50000.0	16666.7	5555.6	1851.9	617.3	205.8	0.0
PIGF	14400.0	4800.0	1600.0	533.3	177.8	59.3	19.8	0.0
EG-VEGF	40000.0	13333.3	4444.4	1481.5	493.8	164.6	54.9	0.0

Note: It is recommended that the researcher dilute their samples so that the reading falls into the standard range.

B. Representation of Standard Curve MFI Values

The values in the tables below are an example of MFI values that can be used to generate a standard curve. These values are for demonstration purposes only. A standard curve must be run with each assay since the MFI values will vary from experiment to experiment.

Group #1: Inflammatory Cytokines

	Eotaxin-2	BLC	RANTES	MCP-1	IL-10	PDGF-BB	IFN- γ	TNF- α
Std 1 (pg/ml)	4000.0	1500.0	1500.0	2000.0	1000.0	1500.0	600.0	1500.0
Standard 1 MFI	6009.0	24723.7	97798.7	9847.0	15214.7	2368.0	5297.0	101153.0
Standard 2 MFI	2423.7	8678.0	48089.3	5934.0	6602.7	804.3	1761.0	35272.7
Standard 3 MFI	1071.7	2714.7	18145.0	3374.7	2754.3	389.0	703.7	13437.3
Standard 4 MFI	562.0	798.3	6658.3	1602.3	1231.3	236.7	351.0	4967.7
Standard 5 MFI	401.7	324.0	2925.3	690.0	552.0	178.7	228.0	1948.3
Standard 6 MFI	352.0	230.0	1722.3	423.3	362.3	165.0	208.7	820.7
Standard 7 MFI	315.0	199.3	1278.0	337.0	270.3	163.0	187.3	443.0
Negative MFI	329.0	184.3	1104.0	279.3	231.0	156.7	179.7	248.7

	IL-5	IL-1 β	IL-6	IL-2	IL-4	G-CSF	IL-12 p70	IL-8
Std 1 (pg/ml)	3000.0	6000.0	6000.0	10000.0	2000.0	10000.0	2000.0	4000.0
Standard 1 MFI	45199.3	18173.7	57493.7	91637.3	83931.0	100348.3	85262.7	106068.3
Standard 2 MFI	30196.7	11164.7	30706.0	52447.3	30105.3	67929.7	26600.0	50432.7
Standard 3 MFI	20200.3	5935.0	10638.7	25330.0	10406.3	33134.0	10460.3	24108.7
Standard 4 MFI	8494.0	2313.0	3407.3	9260.3	3766.3	11542.0	4111.3	13581.0
Standard 5 MFI	3599.3	843.0	1241.7	3655.3	1558.0	3810.3	1620.0	8591.0
Standard 6 MFI	1579.0	391.0	537.0	1451.3	759.0	1346.0	692.3	7060.0
Standard 7 MFI	710.0	214.0	321.0	727.3	485.7	555.3	365.3	6080.3
Negative MFI	158.0	122.3	179.0	303.7	325.3	141.0	189.7	6114.0

	MIP-1 α	IL-7	IL-9	TIMP-2	TNFR1	IL-1Ra	TNFR2	IL-17
Std 1 (pg/ml)	1500.0	1200.0	5000.0	100000.0	30000.0	30000.0	100000.0	5000.0
Standard 1 MFI	39474.3	33697.0	4498.7	16526.7	108109.3	8259.3	10732.7	75865.7
Standard 2 MFI	27111.7	17074.7	1719.7	11053.0	72213.3	2925.3	7483.7	39740.7
Standard 3 MFI	15463.0	5685.7	818.3	6756.0	43000.3	1192.3	6192.0	16753.3
Standard 4 MFI	7771.7	1579.3	515.3	3113.3	16398.0	560.7	3943.7	7000.0
Standard 5 MFI	3133.0	513.3	405.0	1573.7	5254.7	366.0	2034.3	3801.7
Standard 6 MFI	1223.0	279.3	379.3	1042.7	1837.0	301.7	1105.3	2755.7
Standard 7 MFI	569.3	183.7	382.3	797.7	752.3	279.0	692.7	2375.0
Negative MFI	237.3	146.0	359.0	716.3	286.7	258.3	515.0	2016.7

	I-309	IL-12 p40	IL-13	IL-15	Eotaxin-1	IL-16	MIP-1 δ	TNF β
Std 1 (pg/ml)	3000.0	20000.0	1000.0	30000.0	1500.0	30000.0	30000.0	30000.0
Standard 1 MFI	41176.0	84878.7	8915.7	10904.7	10209.3	155631.0	7546.0	93485.0
Standard 2 MFI	9025.3	32176.0	3350.3	3807.7	3575.0	93583.0	4730.0	38730.7
Standard 3 MFI	2699.3	11623.7	1321.7	1280.7	1155.7	43879.3	3477.0	15764.7
Standard 4 MFI	822.3	3915.0	582.0	488.7	447.3	13762.7	2306.0	5896.7
Standard 5 MFI	386.3	1446.7	345.0	280.7	295.0	3951.3	1350.3	2127.3
Standard 6 MFI	277.0	717.3	277.0	202.0	267.3	1333.7	732.3	987.7
Standard 7 MFI	244.3	468.7	243.3	184.0	238.0	621.3	365.3	516.3
Negative MFI	224.0	343.7	230.7	176.3	241.0	358.7	155.3	259.0

	IL-1 α	ICAM-1	IL-11	IFN α	M-CSF	GM-CSF	IL-28A	MIP-3 β
Std 1 (pg/ml)	10000.0	200000.0	100000.0	100000.0	10000.0	30000.0	5000.0	3000.0
Standard 1 MFI	31017.3	17143.0	32176.3	20485.0	80872.7	68617.3	100820.3	13732.7
Standard 2 MFI	20672.3	6429.0	25438.3	19185.3	53481.3	46367.7	27725.0	6677.7
Standard 3 MFI	11137.3	2159.0	18561.3	16773.7	31788.7	25031.0	7971.0	2809.0
Standard 4 MFI	5129.3	745.7	12023.3	10961.7	13604.7	10650.7	2791.3	1118.0
Standard 5 MFI	2257.3	402.0	5813.0	5599.0	5437.3	4057.3	1257.7	578.0
Standard 6 MFI	1406.3	329.3	2392.0	2643.3	2578.7	1631.0	814.7	448.3
Standard 7 MFI	1033.3	281.3	1158.7	1473.7	1451.3	728.0	651.0	384.3
Negative MFI	858.0	276.0	600.7	811.7	805.7	325.0	562.7	356.7

	MIG
Std 1 (pg/ml)	3000.0
Standard 1 MFI	15830.7
Standard 2 MFI	4499.3
Standard 3 MFI	1677.0
Standard 4 MFI	717.3
Standard 5 MFI	454.3
Standard 6 MFI	372.7
Standard 7 MFI	351.7
Negative MFI	327.7

Group #2: Chemokines

	TSLP	Eotaxin-3	MIP-1 β	MDC	I-TAC	TECK	HCC-4	IL-18 Bpa
Std 1 (pg/ml)	2000.0	10000.0	10000.0	10000.0	5000.0	3000.0	5000.0	20000.0
Standard 1 MFI	24577.7	81900.3	40080.3	83774.3	79362.7	11764.7	28546.0	51369.7
Standard 2 MFI	11800.0	28540.3	28365.0	55473.3	39592.3	3749.7	10949.3	41883.0
Standard 3 MFI	6733.0	7138.0	16664.0	30668.0	12458.0	1506.3	5197.0	34907.0
Standard 4 MFI	3426.7	2059.3	7324.7	10491.0	4221.0	842.0	2359.0	16039.0
Standard 5 MFI	1635.0	790.7	2657.0	3540.3	1464.7	625.7	1257.0	6704.3
Standard 6 MFI	776.7	534.3	1136.3	1468.3	925.7	569.3	987.0	2914.0
Standard 7 MFI	469.7	427.0	714.0	626.7	491.7	524.0	843.3	1385.3
Negative MFI	210.0	365.0	589.7	324.0	354.7	509.3	835.0	435.7

	NAP-2	Osteopontin	MIF	LIF	Axl	Betacellulin	CXCL5	Lymphotactin
Std 1 (pg/ml)	200000.0	200000.0	300.0	100000.0	20000.0	30000.0	6000.0	10000.0
Standard 1 MFI	20584.3	41109.7	64017.7	12275.3	9674.0	28426.0	108808.0	12949.3
Standard 2 MFI	14445.3	16759.3	54611.0	5524.0	4009.0	15003.7	76401.0	6320.7
Standard 3 MFI	11780.3	5967.3	28483.7	2793.3	1960.7	5429.7	36097.3	2926.0
Standard 4 MFI	8422.3	1819.0	10862.0	2163.3	1287.0	2363.3	10730.7	1320.0
Standard 5 MFI	4943.3	747.3	4539.3	1858.0	955.0	1093.3	3452.7	709.3
Standard 6 MFI	2521.0	521.7	2060.3	1783.0	894.7	543.3	1465.3	621.3
Standard 7 MFI	1254.7	442.3	1052.0	1694.0	847.0	473.3	728.0	525.7
Negative MFI	584.7	404.7	562.3	1540.3	799.0	381.0	455.0	533.3

	SDF-1 α	MIPF-1	GRO α	PARC	IP-10	MIP-3 α	TARC	MCP-2
Std 1 (pg/ml)	2000.0	10000.0	3000.0	10000.0	15000.0	3000.0	3000.0	1000.0
Standard 1 MFI	17567.3	11265.0	44594.7	13987.0	58851.3	93529.3	12579.7	58280.3
Standard 2 MFI	5244.0	9374.0	29521.7	10605.0	29788.3	44526.0	6028.3	36521.3
Standard 3 MFI	1689.3	8048.3	16467.3	8369.3	16994.7	14211.3	3892.3	19580.0
Standard 4 MFI	735.0	6647.7	8353.3	5374.0	6778.7	4884.0	1476.7	6104.0
Standard 5 MFI	511.3	4123.3	3195.3	2902.3	2488.0	1686.7	699.3	1613.0
Standard 6 MFI	419.0	2394.3	1387.0	1828.7	893.0	850.0	348.7	577.7
Standard 7 MFI	400.3	1232.7	738.7	1211.7	470.3	569.0	222.3	412.7
Negative MFI	366.3	526.7	383.0	940.3	284.0	455.3	168.3	370.0

	MCP-3	GCP-2	HCC-1	IL-31	IL-29	IL-17F	MCP-4	CCL28
Std 1 (pg/ml)	500.0	6000.0	6000.0	100000.0	50000.0	6000.0	3000.0	3000.0
Standard 1 MFI	74339.7	11202.3	59369.0	17442.7	19683.7	14988.7	88911.7	16709.3
Standard 2 MFI	22531.0	4520.3	35707.0	7317.7	10876.0	5540.7	15938.7	4750.0
Standard 3 MFI	6995.7	1956.0	17715.7	3961.0	6276.0	2688.7	5125.0	1895.7
Standard 4 MFI	2510.7	549.3	6020.3	2047.3	2749.3	1282.0	1483.7	1006.7
Standard 5 MFI	1122.7	213.7	2165.0	1112.0	1133.0	694.7	715.0	689.3
Standard 6 MFI	740.0	155.0	976.0	717.7	506.0	516.7	597.0	606.7
Standard 7 MFI	572.3	133.3	637.3	528.3	273.3	438.0	563.0	568.0
Negative MFI	475.7	101.3	460.3	477.3	196.7	371.0	544.7	556.7

	CTACK
Std 1 (pg/ml)	20000.0
Standard 1 MFI	8320.3
Standard 2 MFI	6857.0
Standard 3 MFI	4965.0
Standard 4 MFI	2630.3
Standard 5 MFI	1449.7
Standard 6 MFI	891.7
Standard 7 MFI	722.3
Negative MFI	618.0

Group #3: Cytokines 1

	AgRP	Angiostatin	LAP	IL-2 R α	Angiogenin	PDGF-AB	E-Cadherin	Activin A
Std 1 (pg/ml)	3000.0	40000.0	15000.0	20000.0	10000.0	1000.0	200000.0	80000.0
Standard 1 MFI	109194.3	6254.0	12379.7	66137.0	10435.3	7486.0	6524.3	19351.0
Standard 2 MFI	91862.7	3236.7	5981.7	31108.3	5190.0	2748.0	2467.3	14484.7
Standard 3 MFI	39595.0	2092.3	3538.7	11992.7	2947.0	1196.7	1189.0	10684.3
Standard 4 MFI	15409.0	1585.3	2614.3	4114.7	1900.0	629.0	778.3	6798.0
Standard 5 MFI	5442.7	1511.0	2305.3	1672.7	1171.3	614.3	606.0	1364.0
Standard 6 MFI	2129.0	1440.7	2028.0	887.3	962.3	543.3	576.3	606.3
Standard 7 MFI	869.0	1393.3	1961.0	484.7	780.7	525.3	533.3	466.7
Negative MFI	264.7	1305.3	1876.3	368.3	806.3	485.7	531.3	259.7

	Cripto-1	ICAM-2	IL-2 R β	VEGFR1	Follistatin	Cathepsin S	CD40	Fc γ RIIb/C
Std 1 (pg/ml)	20000.0	350000.0	300000.0	80000.0	3000.0	100000.0	40000.0	180000.0
Standard 1 MFI	68232.3	6983.7	5953.7	8522.3	5299.7	13031.3	25596.3	8937.3
Standard 2 MFI	43175.0	3818.7	4448.0	3286.3	2662.7	11182.7	10222.0	5374.0
Standard 3 MFI	28163.3	2506.3	3008.0	1851.3	1797.3	8539.3	3627.7	3812.3
Standard 4 MFI	9545.7	1633.7	1485.0	1122.7	1419.7	7277.0	1266.7	2486.0
Standard 5 MFI	2593.7	1340.7	642.0	963.7	1290.3	4244.0	578.3	1297.0
Standard 6 MFI	1028.7	1365.3	329.0	873.0	1346.3	2255.0	374.0	1017.0
Standard 7 MFI	712.3	1200.3	257.0	838.3	1271.3	1321.3	274.3	905.3
Negative MFI	524.0	1071.3	152.7	687.7	1139.7	645.3	181.7	666.7

	GP130	Thrombopoietin	DAN	Galectin-7	Fas Ligand	SDF-1 β	VEGF-C	TGF β 2
Std 1 (pg/ml)	400000.0	200000.0	40000.0	180000.0	1000.0	3000.0	40000.0	60000.0
Standard 1 MFI	4813.7	9624.7	8917.3	23395.3	16004.3	8366.0	12806.3	17350.7
Standard 2 MFI	4762.3	2557.3	4867.3	8483.7	6035.7	2771.0	9245.7	12232.7
Standard 3 MFI	3760.3	1061.0	2816.3	4613.7	2435.7	908.7	5171.3	8564.0
Standard 4 MFI	2608.7	395.7	1580.7	1762.3	1070.7	388.7	3226.0	5964.3
Standard 5 MFI	1802.7	256.3	1194.7	966.3	604.3	271.7	2051.3	2557.0
Standard 6 MFI	1359.7	238.0	1058.3	595.7	444.3	250.3	1617.7	982.3
Standard 7 MFI	1337.3	189.7	878.3	435.7	446.3	240.0	1674.7	654.3
Negative MFI	1140.7	165.3	767.7	264.7	306.0	180.0	1223.7	402.7

	IL-13 R α 1	IL-1 R4	Tie-2	TSH	SHH-N	TREM-1	IL-23	TRAIL R4
Std 1 (pg/ml)	80000.0	5000.0	80000.0	150000.0	7000.0	10000.0	100.0	8000.0
Standard 1 MFI	13852.0	54175.3	9293.0	18505.7	7172.3	16710.7	2288.7	8798.7
Standard 2 MFI	8058.7	27986.3	4169.7	11189.0	3910.0	5488.7	1050.0	3310.0
Standard 3 MFI	4046.0	10733.7	1653.7	7014.3	2835.7	2237.7	762.3	1500.3
Standard 4 MFI	2274.0	4309.3	740.7	3445.7	2207.0	1044.7	693.3	833.3
Standard 5 MFI	1456.3	1783.3	451.7	1697.3	1906.0	595.0	632.7	597.7
Standard 6 MFI	1167.7	774.0	385.7	875.3	2358.0	501.0	755.0	612.0
Standard 7 MFI	1134.7	477.3	346.0	651.7	2533.3	438.7	766.7	605.3
Negative MFI	892.3	252.3	267.7	453.0	1510.0	342.7	597.7	469.3

Group #4: Cytokines 2

	AFP	ANGPTL4	CA125	FSH	hCGβ	IGF-1R	IL-1 RII	IL-18 Rβ
Std 1 (pg/ml)	150000.0	5000.0	100000.0	150000.0	500.0	100000.0	50000.0	3000.0
Standard 1 MFI	8218.7	4732.3	10033.7	13087.7	1987.7	32848.7	48599.0	80093.3
Standard 2 MFI	7052.3	2379.7	3921.0	13376.7	597.7	13155.3	22329.7	34199.0
Standard 3 MFI	5480.3	1202.0	1786.7	10335.7	308.0	5281.7	7794.7	10532.0
Standard 4 MFI	3408.7	711.7	902.7	6718.0	221.3	1981.3	2859.3	3636.0
Standard 5 MFI	1632.3	537.0	550.7	3166.0	191.0	810.3	1026.7	1409.7
Standard 6 MFI	798.0	458.0	471.7	1606.7	160.3	395.7	516.3	568.3
Standard 7 MFI	501.7	455.3	402.0	850.3	154.7	266.0	334.3	339.3
Negative MFI	320.0	406.3	359.0	438.7	151.7	181.0	269.7	168.3

	IL-21	IL-3	Leptin	MMP-10	MMP-13	MMP-3	MMP-8	NSE
Std 1 (pg/ml)	50000.0	10000.0	5000.0	10000.0	5000.0	150000.0	150000.0	180000.0
Standard 1 MFI	3746.3	1698.3	7333.3	19906.7	73487.7	47550.0	11102.7	14138.3
Standard 2 MFI	1176.3	605.0	3604.7	8576.0	32003.0	33328.7	8245.0	8376.7
Standard 3 MFI	444.3	319.7	1712.0	2978.7	8302.3	16061.3	4389.7	3986.0
Standard 4 MFI	298.0	237.0	991.3	1082.0	2012.3	6994.7	2215.7	1501.7
Standard 5 MFI	185.7	204.7	630.7	507.0	556.7	3255.0	1182.0	615.7
Standard 6 MFI	154.7	173.0	486.0	292.0	254.7	2208.7	795.0	344.0
Standard 7 MFI	160.3	166.3	492.7	211.3	168.0	1647.3	705.7	254.3
Negative MFI	139.3	159.7	419.0	170.7	125.0	1465.3	582.3	188.0

	OSM	Prolactin	PSA-Free	Siglec-9	TACE	TIMP-4
Std 1 (pg/ml)	20000.0	150000.0	200000.0	20000.0	200000.0	10000.0
Standard 1 MFI	3281.7	13139.7	10096.3	44088.3	11882.3	17347.3
Standard 2 MFI	1192.0	8514.3	5909.3	30732.7	4839.3	6507.3
Standard 3 MFI	553.3	4845.3	2359.7	12453.3	2065.7	2154.3
Standard 4 MFI	374.0	2416.0	750.3	4734.3	919.0	736.3
Standard 5 MFI	256.0	1132.3	320.0	1832.7	729.0	358.0
Standard 6 MFI	207.3	462.0	194.3	738.0	547.0	256.7
Standard 7 MFI	205.3	317.3	164.3	404.0	528.7	214.7
Negative MFI	186.0	193.3	130.0	200.3	526.0	207.7

Group #5: Receptors

	TRAIL R3	DR6	CD14	CEACAM-1	IL-1 R1	PECAM-1	E-Selectin	CD105
Std 1 (pg/ml)	100000.0	85000.0	90000.0	100000.0	80000.0	100000.0	100000.0	33300.0
Standard 1 MFI	18814.3	28881.3	14453.7	10227.3	17817.3	23933.3	19241.3	27467.3
Standard 2 MFI	13788.3	16729.7	5621.7	3777.7	13406.3	17757.7	9699.7	19653.7
Standard 3 MFI	9884.0	8151.0	2809.7	1508.0	8733.7	12764.0	4179.7	12583.0
Standard 4 MFI	5823.7	3661.3	1412.3	1032.0	4382.0	7155.3	1776.7	6677.7
Standard 5 MFI	3483.7	1965.0	856.7	588.3	1878.7	3308.0	1073.0	3351.0
Standard 6 MFI	2221.7	1197.7	682.3	653.7	785.3	1493.0	859.3	1770.3
Standard 7 MFI	1815.7	1011.3	657.0	546.3	415.7	817.0	801.7	1164.7
Negative MFI	1524.3	799.0	581.0	533.3	148.0	430.3	677.7	801.3

	Trappin-2	ErbB3	CD30	IL-2 Ry	CD80	Contactin-2	uPAR	Dtk
Std 1 (pg/ml)	100000.0	80000.0	100000.0	30000.0	60000.0	100000.0	100000.0	100000.0
Standard 1 MFI	16045.7	42796.7	30263.3	128193.0	12167.3	16744.0	17813.0	13473.0
Standard 2 MFI	17089.0	26639.0	20415.0	27735.3	16192.3	7214.3	7593.3	7387.0
Standard 3 MFI	17935.3	15030.3	12202.3	8156.0	11934.7	2227.3	3378.7	3396.7
Standard 4 MFI	15563.3	6786.0	6442.3	2985.7	5909.3	672.7	1479.3	1483.7
Standard 5 MFI	9739.7	2999.3	2913.3	1642.3	2756.3	339.7	654.7	775.0
Standard 6 MFI	5228.0	1608.3	1665.0	1184.0	1271.7	240.3	434.3	535.0
Standard 7 MFI	2719.3	1212.3	1131.7	1052.7	1082.7	241.3	374.0	425.0
Negative MFI	906.3	838.7	634.7	864.7	585.3	170.7	267.0	327.3

	ALCAM	LIMP2	MICA	TIM-1	IL-17 RA	XEDAR	Fas	IL-21 R
Std 1 (pg/ml)	100000.0	100000.0	90000.0	100000.0	100000.0	150000.0	50000.0	100000.0
Standard 1 MFI	23726.7	50792.0	32682.0	19691.0	19300.7	73795.7	184211.7	11149.7
Standard 2 MFI	18480.7	17973.7	17337.3	13568.3	13077.0	39932.0	59792.3	3970.0
Standard 3 MFI	12449.0	7199.0	8761.7	9943.7	8267.0	24817.7	17203.3	1472.3
Standard 4 MFI	5698.7	2689.0	3809.7	5887.3	4039.7	11385.3	5262.7	616.3
Standard 5 MFI	2731.3	1035.7	1674.3	2925.3	1831.0	5045.0	2104.7	354.7
Standard 6 MFI	1611.0	448.7	707.3	1274.0	770.7	2390.7	1050.3	249.7
Standard 7 MFI	1299.7	278.0	418.7	674.0	500.3	1517.0	749.3	226.3
Negative MFI	1015.3	148.0	207.7	290.0	267.7	754.3	529.7	175.0

	CD40 ligand	Flt-3 ligand	GITR
Std 1 (pg/ml)	10000.0	50000.0	100000.0
Standard 1 MFI	163724.3	11440.7	7874.7
Standard 2 MFI	68268.0	8734.3	3903.7
Standard 3 MFI	32980.0	7407.0	2035.0
Standard 4 MFI	9971.0	5718.0	1076.0
Standard 5 MFI	3499.7	3578.7	688.7
Standard 6 MFI	1312.3	1807.3	558.0
Standard 7 MFI	803.3	889.7	524.7
Negative MFI	353.7	293.0	455.7

Group #6: Growth Factors

	GDF-15	OPG	VEGF-D	β -NGF	Growth Hormone	IGF-1	NGFR	TGF- β 3
Std 1 (pg/ml)	11110.0	3000.0	33330.0	10000.0	80000.0	150000.0	100000.0	100000.0
Standard 1 MFI	6236.7	10007.7	202900.3	26533.7	7750.7	7045.0	7791.0	18808.0
Standard 2 MFI	5346.0	3545.7	165420.3	22643.7	5827.7	4253.3	5113.3	14642.7
Standard 3 MFI	4883.3	1469.0	55935.7	16992.7	4597.3	1948.0	2560.0	11788.3
Standard 4 MFI	3944.0	848.3	16368.0	9026.0	3240.7	795.7	1158.7	6338.7
Standard 5 MFI	2052.7	590.0	5820.0	3630.7	1674.0	401.7	549.7	2429.0
Standard 6 MFI	962.7	489.7	2012.3	1341.7	689.0	287.7	325.3	812.0
Standard 7 MFI	512.0	438.7	650.3	442.3	285.0	209.0	241.3	331.7
Negative MFI	380.3	494.3	79.7	52.3	104.0	253.7	245.7	132.7

	BMP-4	IGFBP-2	BMP-7	VEGF-A	bFGF	TGF β 1	SCF	IGFBP-6
Std 1 (pg/ml)	20000.0	100000.0	100000.0	3000.0	3000.0	200000.0	25000.0	100000.0
Standard 1 MFI	73036.7	15704.3	3777.7	3639.7	109342.3	5395.7	82845.0	13867.3
Standard 2 MFI	59799.3	7312.3	1360.3	1652.0	32355.0	3561.3	52824.3	6879.7
Standard 3 MFI	34980.3	3240.7	560.3	733.3	5665.0	1952.7	23764.3	3257.7
Standard 4 MFI	17305.3	1456.7	348.3	440.0	1600.7	889.0	9573.3	1806.0
Standard 5 MFI	5586.3	762.7	279.7	314.7	590.7	389.0	3572.3	1211.3
Standard 6 MFI	1787.7	490.3	232.0	262.0	258.7	182.7	1352.0	984.7
Standard 7 MFI	503.7	407.3	205.7	215.3	134.3	93.3	551.0	857.3
Negative MFI	104.3	415.7	231.7	265.0	101.7	74.3	171.0	994.7

	VEGFR3	VEGFR2	PIGF	EG-VEGF
Std 1 (pg/ml)	100000.0	150000.0	14400.0	40000.0
Standard 1 MFI	6821.3	18265.3	37862.7	26271.0
Standard 2 MFI	3089.3	8750.3	26714.0	16525.0
Standard 3 MFI	1236.7	3515.7	21317.3	7571.3
Standard 4 MFI	596.3	1708.0	14647.7	2987.7
Standard 5 MFI	387.7	883.3	7832.7	943.3
Standard 6 MFI	312.3	604.7	3229.3	393.7
Standard 7 MFI	266.7	503.3	1131.3	245.3
Negative MFI	295.7	559.0	305.3	229.7

C. Lower Limit of Detection (LOD) of Target Proteins

The LOD is the minimum detectable concentration (i.e., sensitivity) of the assay. It was determined by spiking each purified protein standard into RayBio® Human Serum/Plasma Diluent (Item 10).

Group #1: Inflammatory Cytokines

Target	LOD (pg/mL)
Eotaxin-2	46.2
BLC	6.7
RANTES	1.7
MCP-1	2.6
IL-10	1.0
PDGF-BB	48.7
IFN- γ	10.3
TNF- α	0.7
IL-5	0.4
IL-1 β	2.3
IL-6	5.3
IL-2	2.0
IL-4	1.3
G-CSF	2.3
IL-12 p70	1.0
IL-8	11.4
MIP-1 α	0.6
IL-7	1.9
IL-9	93.1
TIMP-2	98.0
TNFR1	9.8
IL-1Ra	215.1
TNFR2	61.3
IL-17	12.3
I-309	18.4
IL-12 p40	20.4
IL-13	3.4
IL-15	145.1
Eotaxin-1	17.7
IL-16	17.6
MIP-1 δ	1.3
TNF β	14.6
IL-1 α	15.6
ICAM-1	570.1
IL-11	84.0
IFN α	28.6
M-CSF	2.7
GM-CSF	5.2
IL-28A	27.1
MIP-3 β	8.6
MIG	20.2

Group #2: Chemokines

Target	LOD (pg/mL)
TSLP	0.3
Eotaxin-3	53.1
MIP-1 β	16.0
MDC	6.4
I-TAC	14.3
TECK	47.4
HCC-4	35.0
IL-18 Bpa	16.7
NAP-2	55.9
Osteopontin	938.9
MIF	0.1
LIF	1904.3
Axl	230.5
Betacellulin	100.6
CXCL5	11.3
Lymphotactin	126.1
SDF-1 α	39.9
MPIF-1	4.6
GRO α	1.1
PARC	10.2
IP-10	14.1
MIP-3 α	10.7
TARC	7.3
MCP-2	4.2
MCP-3	2.5
GCP-2	41.6
HCC-1	5.8
IL-31	91.9
IL-29	36.1
IL-17F	10.7
MCP-4	36.7
CCL28	68.5
CTACK	19.3

Group #3: Cytokines 1

Target	LOD (pg/mL)
AgRP	8.1
Angiostatin	417.5
LAP	124.5
IL-2 R α	13.8
Angiogenin	8.7
PDGF-AB	12.5
E-Cadherin	2697.0
Activin A	774.3
Cripto-1	63.5
ICAM-2	1137.2
IL-2 R β	313.3
VEGFR1	421.3
Follistatin	33.1
Cathepsin S	47.6
CD40	63.1
Fc γ RIIB/C	123.3
GP130	192.1
Thrombopoietin	2194.3
DAN	35.1
Galectin-7	44.2
Fas Ligand	3.2
SDF-1 β	28.9
VEGF-C	29.0
TGF β 2	46.9
IL-13 R α 1	144.8
IL-1 R4	0.8
Tie-2	225.4
TSH	172.4
SHH-N	144.4
TREM-1	112.9
IL-23	2.3
TRAIL R4	28.2

Group #4: Cytokines 2

Target	LOD (pg/mL)
AFP	51.5
ANGPTL4	42.8
CA125	924.8
FSH	39.5
hCG β	2.0
IGF-1R	96.3
IL-1 RII	81.8
IL-18 R β	1.1
IL-21	670.3
IL-3	311.2
Leptin	16.3
MMP-10	16.8
MMP-13	4.8
MMP-3	170.9
MMP-8	169.4
NSE	0.0
OSM	128.4
Prolactin	66.0
PSA-Free	671.0
Siglec-9	6.5
TACE	1583.7
TIMP-4	25.9

Group #5: Receptors

Target	LOD (pg/mL)
TRAIL R3	186.29
DR6	181.06
CD14	278.24
CEACAM-1	510.16
IL-1 R1	13.49
PECAM-1	13.76
E-Selectin	203.26
CD105	15.02
Trappin-2	8.62
ErbB3	68.52
CD30	62.97
IL-2 Ry	196.05
CD80	54.75
Contactin-2	821.92
uPAR	733.72
Dtk	394.52
ALCAM	188.69
LIMP2	197.20
MICA	21.86
TIM-1	26.99
IL-17 RA	41.84
XEDAR	126.77
Fas	194.08
IL-21 R	491.66
CD40 ligand	25.32
Flt-3 ligand	17.76
GITR	391.13

Group #6: Growth Factors

Target	LOD (pg/mL)
GDF-15	9.48
OPG	28.24
VEGF-D	7.92
β -NGF	0.92
Growth Hormone	25.42
IGF-1	1268.76
NGFR	312.27
TGF- β 3	105.98
BMP-4	4.36
IGFBP-2	272.01
BMP-7	1328.98
VEGF-A	24.48
bFGF	13.04
TGF β 1	232.19
SCF	3.09
IGFBP-6	992.44
VEGFR3	181.93
VEGFR2	508.23
PIGF	4.38
EG-VEGF	49.64

NOTE: The LOD may vary from run-to-run and batch-to-batch.

D. Cross Reactivity

No detectable or significant cross reactivity was detected between antibodies or targets.

E. Percent (%) Recovery of Spiked Standards

For spike recovery of standards, target proteins with concentrations corresponding to standard 2 (Std2) were spiked into RayBio® Human Serum/Plasma Diluent (Item 10) across 9 replicates. The spiked proteins were assayed and the measured concentrations were compared to the expected concentrations.

Group #1: Inflammatory Cytokines

Target	Percent Recovery
Eotaxin-2	98.4%
BLC	99.3%
RANTES	100.3%
MCP-1	97.4%
IL-10	99.2%
PDGF-BB	92.9%
IFN- γ	96.4%
TNF- α	99.6%
IL-5	98.9%
IL-1 β	99.2%
IL-6	100.6%
IL-2	100.6%
IL-4	100.2%
G-CSF	102.3%
IL-12 p70	96.6%
IL-8	100.8%
MIP-1 α	99.8%
IL-7	99.9%
IL-9	96.5%
TIMP-2	98.7%
TNFR1	102.6%
IL-1Ra	98.0%
TNFR2	77.5%
IL-17	100.4%
I-309	97.5%
IL-12 p40	100.0%
IL-13	99.5%
IL-15	99.6%
Eotaxin-1	99.0%
IL-16	101.4%
MIP-1 δ	87.1%
TNF β	100.3%
IL-1 α	100.8%
ICAM-1	99.7%
IL-11	99.3%
IFN α	118.3%
M-CSF	99.2%
GM-CSF	102.3%
IL-28A	99.3%
MIP-3 β	99.6%
MIG	97.2%

Group #2: Chemokines

Target	Percent Recovery
TSLP	97.5%
Eotaxin-3	100.4%
MIP-1 β	98.6%
MDC	98.6%
I-TAC	97.2%
TECK	100.6%
HCC-4	100.3%
IL-18 Bpa	100.0%
NAP-2	100.5%
Osteopontin	105.5%
MIF	97.5%
LIF	100.4%
Axl	98.6%
Betacellulin	98.6%
CXCL5	97.2%
Lymphotactin	100.6%
SDF-1 α	100.3%
MIPF-1	100.0%
GRO α	100.5%
PARC	105.5%
IP-10	97.5%
MIP-3 α	100.4%
TARC	98.6%
MCP-2	98.6%
MCP-3	97.2%
GCP-2	100.6%
HCC-1	100.3%
IL-31	100.0%
IL-29	100.5%
IL-17F	105.5%
MCP-4	97.5%
CCL28	100.4%
CTACK	98.6%

Group #3: Cytokines 1

Target	Percent Recovery
AgRP	89.2%
Angiostatin	86.4%
LAP	90.0%
IL-2 R α	100.8%
Angiogenin	93.2%
PDGF-AB	98.5%
E-Cadherin	95.8%
Activin A	88.6%
Cripto-1	111.0%
ICAM-2	99.3%
IL-2 R β	88.0%
VEGFR1	97.7%
Follistatin	93.5%
Cathepsin S	99.5%
CD40	99.9%
Fc γ RIIB/C	119.4%
GP130	104.8%
Thrombopoietin	93.8%
DAN	99.6%
Galectin-7	100.2%
Fas Ligand	98.7%
SDF-1 β	99.1%
VEGF-C	101.1%
TGF β 2	91.1%
IL-13 R α 1	100.3%
IL-1 R4	100.3%
Tie-2	100.1%
TSH	95.5%
SHH-N	103.7%
TREM-1	94.3%
IL-23	88.4%
TRAIL R4	99.0%

Group #4: Cytokines 2

Target	Percent Recovery
AFP	105.5%
ANGPTL4	98.7%
CA125	97.3%
FSH	95.9%
hCG β	86.9%
IGF-1R	100.3%
IL-1 RII	100.0%
IL-18 R β	100.2%
IL-21	95.8%
IL-3	86.3%
Leptin	98.9%
MMP-10	100.0%
MMP-13	99.9%
MMP-3	101.3%
MMP-8	100.3%
NSE	99.8%
OSM	96.3%
Prolactin	100.5%
PSA-Free	99.7%
Siglec-9	99.7%
TACE	96.9%
TIMP-4	99.2%

Group #5: Receptors

Target	Percent Recovery
TRAIL R3	104.1%
DR6	100.0%
CD14	100.3%
CEACAM-1	96.1%
IL-1 R1	105.4%
PECAM-1	103.1%
E-Selectin	100.3%
CD105	103.2%
Trappin-2	99.6%
ErbB3	104.5%
CD30	109.7%
IL-2 Ry	98.9%
CD80	84.3%
Contactin-2	99.0%
uPAR	100.1%
Dtk	100.2%
ALCAM	101.4%
LIMP2	100.6%
MICA	100.4%
TIM-1	103.3%
IL-17 RA	109.3%
XEDAR	99.0%
Fas	100.5%
IL-21 R	100.0%
CD40 ligand	100.2%
Flt-3 ligand	93.3%
GITR	100.4%

Group #6: Growth Factors

Target	Percent Recovery
GDF-15	100%
OPG	107%
VEGF-D	98%
β -NGF	93%
Growth Hormone	101%
IGF-1	101%
NGFR	100%
TGF- β 3	86%
BMP-4	101%
IGFBP-2	102%
BMP-7	113%
VEGF-A	103%
bFGF	110%
TGF β 1	101%
SCF	103%
IGFBP-6	100%
VEGFR3	103%
VEGFR2	103%
PIGF	105%
EG-VEGF	102%

F. Linearity of Dilution

To test the linearity of dilution, samples (n = 12) were serially diluted in RayBio® Human Serum/Plasma Diluent (Item 10) 1:3, 1:9, and 1:27. The measured concentrations of serially diluted samples were compared to that of the undiluted samples and averaged. See data below.

Group #1: Inflammatory Cytokines

Target	Linearity of Dilution
Eotaxin-2	100.3%
BLC	91.4%
RANTES	103.0%
MCP-1	100.9%
IL-10	101.6%
PDGF-BB	119.8%
IFN- γ	109.1%
TNF- α	102.5%
IL-5	94.8%
IL-1 β	99.8%
IL-6	102.7%
IL-2	100.1%
IL-4	102.5%
G-CSF	100.8%
IL-12 p70	113.8%
IL-8	100.5%
MIP-1 α	116.8%
IL-7	99.9%
IL-9	108.2%
TIMP-2	99.5%
TNFR1	100.7%
IL-1Ra	104.1%
TNFR2	101.7%
IL-17	103.1%
I-309	116.1%
IL-12 p40	101.2%
IL-13	92.9%
IL-15	100.0%
Eotaxin-1	100.6%
IL-16	99.6%
MIP-1 δ	109.2%
TNF β	102.3%
IL-1 α	100.5%
ICAM-1	98.4%
IL-11	100.0%
IFN α	93.8%
M-CSF	99.6%
GM-CSF	99.4%
IL-28A	105.9%
MIP-3 β	99.7%
MIG	112.2%

Group #2: Chemokines

Target	Linearity of Dilution
TSLP	104.2%
Eotaxin-3	97.1%
MIP-1 β	99.0%
MDC	102.4%
I-TAC	100.3%
TECK	97.9%
HCC-4	101.1%
IL-18 Bpa	117.6%
NAP-2	90.3%
Osteopontin	98.0%
MIF	96.0%
LIF	102.0%
Axl	104.0%
Betacellulin	99.1%
CXCL5	97.9%
Lymphotactin	99.9%
SDF-1 α	101.3%
MPIF-1	110.5%
GRO α	101.7%
PARC	99.4%
IP-10	98.8%
MIP-3 α	104.0%
TARC	97.7%
MCP-2	96.6%
MCP-3	103.2%
GCP-2	101.8%
HCC-1	93.2%
IL-31	102.6%
IL-29	100.1%
IL-17F	106.7%
MCP-4	101.7%
CCL28	110.2%
CTACK	98.7%

Group #3: Cytokines 1

Target	Linearity of Dilution
AgRP	104.9%
Angiostatin	97.6%
LAP	116.5%
IL-2 R α	100.0%
Angiogenin	110.7%
PDGF-AB	99.3%
E-Cadherin	110.2%
Activin A	99.8%
Cripto-1	104.7%
ICAM-2	98.2%
IL-2 R β	105.1%
VEGFR1	106.6%
Follistatin	113.9%
Cathepsin S	93.5%
CD40	99.3%
Fc γ RIIB/C	109.4%
GP130	81.5%
Thrombopoietin	121.5%
DAN	99.7%
Galectin-7	107.2%
Fas Ligand	100.6%
SDF-1 β	98.1%
VEGF-C	98.7%
TGF β 2	100.6%
IL-13 R α 1	99.0%
IL-1 R4	100.3%
Tie-2	100.1%
TSH	100.8%
SHH-N	73.8%
TREM-1	104.2%
IL-23	103.2%
TRAIL R4	102.0%

Group #4: Cytokines 2

Target	Linearity of Dilution
AFP	97.7%
ANGPTL4	94.1%
CA125	102.1%
FSH	86.3%
hCG β	128.9%
IGF-1R	101.0%
IL-1 RII	101.6%
IL-18 R β	103.8%
IL-21	111.0%
IL-3	127.2%
Leptin	99.0%
MMP-10	99.7%
MMP-13	102.1%
MMP-3	102.4%
MMP-8	101.0%
NSE	99.9%
OSM	101.1%
Prolactin	89.9%
PSA-Free	96.9%
Siglec-9	97.3%
TACE	100.2%
TIMP-4	82.7%

Group #5: Receptors

Target	Linearity of Dilution
TRAIL R3	100.5%
DR6	93.3%
CD14	102.2%
CEACAM-1	104.9%
IL-1 R1	99.6%
PECAM-1	104.9%
E-Selectin	99.7%
CD105	100.8%
Trappin-2	108.5%
ErbB3	100.5%
CD30	105.5%
IL-2 Ry	108.9%
CD80	111.9%
Contactin-2	100.3%
uPAR	102.8%
Dtk	101.0%
ALCAM	88.4%
LIMP2	104.1%
MICA	101.2%
TIM-1	102.7%
IL-17 RA	102.0%
XEDAR	94.0%
Fas	102.7%
IL-21 R	101.3%
CD40 ligand	99.3%
Flt-3 ligand	100.1%
GITR	99.4%

Group #6: Growth Factors

Target	Linearity of Dilution
GDF-15	100.3%
OPG	106.6%
VEGF-D	97.9%
β -NGF	93.4%
Growth Factor	100.8%
IGF-1	100.6%
NGFR	100.1%
TGF- β 3	86.1%
BMP-4	101.3%
IGFBP-2	101.8%
BMP-7	112.6%
VEGF-A	102.5%
bFGF	110.2%
TGF β 1	100.9%
SCF	103.2%
IGFBP-6	100.4%
VEGFR3	102.6%
VEGFR2	102.8%
PIGF	105.1%
EG-VEGF	101.7%

G.Intra- and Inter-Assay Precision

1) Intra-Assay Precision

Spiked standard protein in RayBio® Human Serum/Plasma Diluent (Item 10) was analyzed in triplicate. Data was chosen from one of three replicates for each target as indicated at the concentrations of protein standard 2 (Sample #1) and 3 (Sample #2) respectively. The intra-assay precision, or coefficient of variation (CV), of the samples is provided below.

Group #1: Inflammatory Cytokines

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
Eotaxin-2	1	1333.7	23.0	1.7%
	2	443.4	2.8	0.6%
BLC	1	499.4	14.1	2.8%
	2	169.2	2.6	1.5%
RANTES	1	501.2	56.5	11.3%
	2	166.1	6.0	3.6%
MCP-1	1	645.2	48.3	7.5%
	2	236.2	10.8	4.6%
IL-10	1	333.3	16.9	5.1%
	2	110.9	6.9	6.2%
PDGF-BB	1	476.3	26.6	5.6%
	2	189.6	5.3	2.8%
IFN- γ	1	196.3	4.7	2.4%
	2	70.0	3.9	5.6%
TNF- α	1	491.3	35.3	7.2%
	2	175.5	2.0	1.1%
IL-5	1	748.1	49.0	6.5%
	2	379.2	43.7	11.5%
IL-1 β	1	1959.9	12.4	0.6%
	2	692.6	13.1	1.9%
IL-6	1	2010.7	270.9	13.5%
	2	658.7	23.7	3.6%
IL-2	1	3304.9	100.0	3.0%
	2	1145.1	179.2	15.7%
IL-4	1	667.2	15.1	2.3%
	2	220.5	12.5	5.7%
G-CSF	1	3346.0	387.4	11.6%
	2	1116.3	51.3	4.6%
IL-12 p70	1	637.6	4.0	0.6%
	2	253.0	9.9	3.9%
IL-8	1	1336.0	123.6	9.3%
	2	439.1	18.4	4.2%
MIP-1 α	1	504.3	38.6	7.6%
	2	163.6	10.7	6.5%
IL-7	1	400.6	35.5	8.9%
	2	133.4	6.2	4.7%
IL-9	1	1635.5	66.8	4.1%
	2	568.6	60.7	10.7%
TIMP-2	1	32245.6	900.4	2.8%
	2	11718.9	505.2	4.3%
TNFR1	1	9654.7	1352.7	14.0%
	2	3514.1	246.7	7.0%

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
IL-1Ra	1	9884.0	36.8	0.4%
	2	3453.9	127.4	3.7%
TNFR2	1	24509.8	3117.7	12.7%
	2	15170.2	2368.9	15.6%
IL-17	1	1669.4	105.2	6.3%
	2	554.4	14.1	2.5%
I-309	1	979.7	28.3	2.9%
	2	391.0	19.1	4.9%
IL-12 p40	1	6656.9	567.5	8.5%
	2	2258.0	87.7	3.9%
IL-13	1	333.0	4.6	1.4%
	2	111.9	3.7	3.3%
IL-15	1	10006.4	314.2	3.1%
	2	3313.7	85.2	2.6%
Eotaxin-1	1	500.0	21.6	4.3%
	2	166.9	7.6	4.6%
IL-16	1	8602.5	694.6	8.1%
	2	3382.2	130.0	3.8%
MIP-1 δ	1	8533.7	872.7	10.2%
	2	3839.1	409.6	10.7%
TNF β	1	9973.3	1219.5	12.2%
	2	3415.3	44.5	1.3%
IL-1 α	1	3333.8	142.4	4.3%
	2	1112.4	40.0	3.6%
ICAM-1	1	66591.9	1577.4	2.4%
	2	22538.8	284.1	1.3%
IL-11	1	33314.4	6489.3	19.5%
	2	11089.0	1150.4	10.4%
IFN α	1	21326.7	1865.2	8.7%
	2	11774.1	1071.8	9.1%
M-CSF	1	3244.0	350.1	10.8%
	2	1168.1	105.1	9.0%
GM-CSF	1	21326.7	1865.2	8.7%
	2	3344.5	168.4	5.0%
IL-28A	1	1660.6	95.1	5.7%
	2	558.9	24.8	4.4%
MIP-3 β	1	998.4	37.4	3.7%
	2	337.4	5.3	1.6%
MIG	1	975.4	27.4	2.8%
	2	374.3	13.9	3.7%

Group #2: Chemokines

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
TSLP	1	643.9	38.1	5.9%
	2	237.4	21.4	9.0%
Eotaxin-3	1	3334.9	274.7	8.2%
	2	1093.8	67.9	6.2%
MIP-1 β	1	3345.2	121.8	3.6%
	2	1110.9	165.1	14.9%
MDC	1	3339.7	282.3	8.5%
	2	1113.5	68.7	6.2%
I-TAC	1	1670.7	160.9	9.6%
	2	542.4	16.7	3.1%
TECK	1	997.2	93.1	9.3%
	2	336.0	24.6	7.3%
HCC-4	1	1638.1	133.7	8.2%
	2	593.8	31.2	5.3%
IL-18 Bpa	1	6310.3	122.5	1.9%
	2	2911.4	109.6	3.8%
NAP-2	1	43737.1	9407.4	21.5%
	2	22908.2	1931.8	8.4%
Osteopontin	1	66823.4	4690.6	7.0%
	2	21583.1	1811.9	8.4%
MIF	1	100.1	7.7	7.7%
	2	33.4	0.8	2.5%
LIF	1	33787.3	6017.1	17.8%
	2	10003.5	1030.2	10.3%
Axl	1	6644.9	96.6	1.5%
	2	2212.9	113.5	5.1%
Betacellulin	1	8028.2	1185.2	8.4%
	2	3235.4	270.3	11.1%
CXCL5	1	1699.3	304.2	17.9%
	2	665.4	48.9	7.3%
Lymphotactin	1	3326.7	196.0	5.9%
	2	1137.8	173.4	15.2%
SDF-1 α	1	666.0	35.6	5.3%
	2	221.2	5.6	2.5%
MPIF-1	1	2647.9	30.7	1.2%
	2	949.7	151.6	16.0%
GRO α	1	1007.7	116.5	11.6%
	2	323.5	38.3	11.8%
PARC	1	3132.4	235.6	7.5%
	2	1077.4	60.3	5.6%

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
IP-10	1	4832.1	79.7	1.6%
	2	1837.4	82.0	4.5%
MIP-3 α	1	1001.1	40.8	4.1%
	2	329.4	9.6	2.9%
TARC	1	1005.8	141.9	14.1%
	2	331.4	17.6	5.3%
MCP-2	1	326.8	23.6	7.2%
	2	115.4	3.2	2.7%
MCP-3	1	164.7	8.8	5.4%
	2	58.6	2.4	4.0%
GCP-2	1	1983.5	39.4	2.0%
	2	707.2	42.4	6.0%
HCC-1	1	1990.0	313.6	15.8%
	2	690.9	78.1	11.3%
IL-31	1	31458.7	1556.3	4.9%
	2	12045.9	512.5	4.3%
IL-29	1	16464.2	1248.8	7.6%
	2	5822.0	132.1	2.3%
IL-17F	1	1902.7	82.2	4.3%
	2	751.0	49.6	6.6%
MCP-4	1	990.3	14.6	1.5%
	2	374.6	11.3	3.0%
CCL28	1	971.3	24.5	2.5%
	2	382.1	11.1	2.9%
CTACK	1	6681.5	1054.5	15.8%
	2	2280.9	174.6	7.7%

Group #3: Cytokines 1

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
AgRP	1	842.2	151.9	18.0%
	2	315.6	22.5	7.1%
Angiostatin	1	12570.9	1501.3	11.9%
	2	4398.0	537.2	12.2%
LAP	1	4848.5	154.3	3.2%
	2	1854.4	293.1	15.8%
IL-2 R α	1	6687.4	1046.3	15.6%
	2	2233.2	162.4	7.3%
Angiogenin	1	3223.0	277.9	8.6%
	2	1155.3	130.3	11.3%
PDGF-AB	1	328.4	19.5	5.9%
	2	112.2	1.5	1.3%
E-Cadherin	1	65675.6	3813.3	5.8%
	2	22815.3	786.7	3.4%
Activin A	1	21166.4	767.3	3.6%
	2	8937.6	1271.9	14.2%
Cripto-1	1	5830.7	479.7	8.2%
	2	2561.0	122.3	4.8%
ICAM-2	1	111395.7	12511.5	11.2%
	2	42473.2	6188.5	14.6%
IL-2 R β	1	96545.2	5458.2	5.7%
	2	35532.5	6765.4	19.0%
VEGFR1	1	24361.9	787.9	3.2%
	2	10400.7	1355.2	13.0%
Follistatin	1	885.3	137.1	15.5%
	2	366.8	83.1	22.6%
Cathepsin S	1	29676.7	6202.1	20.9%
	2	7768.0	503.8	6.5%
CD40	1	13351.7	1086.3	8.1%
	2	4424.5	155.6	3.5%
Fc γ RIIB/C	1	54017.4	4830.4	8.9%
	2	23776.4	1738.0	7.3%
GP130	1	71550.0	20505.9	28.7%
	2	34694.6	6487.6	18.7%
Thrombopoietin	1	63574.1	3281.3	5.2%
	2	28164.7	466.7	1.7%
DAN	1	13400.3	673.4	5.0%
	2	4579.8	448.9	9.8%
Galectin-7	1	62245.7	5001.7	8.0%
	2	30334.0	6589.7	21.7%

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
Fas Ligand	1	330.0	18.0	5.5%
	2	112.5	13.1	11.6%
SDF-1 β	1	1002.7	22.0	2.2%
	2	322.9	16.1	5.0%
VEGF-C	1	11205.9	1049.9	9.4%
	2	4817.2	317.8	6.6%
TGF β 2	1	14738.8	320.3	2.2%
	2	6370.5	826.3	13.0%
IL-13 R α 1	1	27144.7	4214.3	15.5%
	2	8492.5	987.5	11.6%
IL-1 R4	1	1679.7	180.6	10.7%
	2	535.8	16.4	3.1%
Tie-2	1	26779.7	915.7	3.4%
	2	8668.0	499.4	5.8%
TSH	1	46873.6	2255.0	4.8%
	2	18822.6	2308.4	12.3%
SHH-N	1	1299.6	325.4	25.0%
	2	615.1	72.7	11.8%
TREM-1	1	3214.9	125.8	3.9%
	2	1217.5	26.7	2.2%
IL-23	1	31.4	10.6	33.8%
	2	12.0	3.1	25.5%
TRAIL R4	1	2603.5	212.9	8.2%
	2	921.3	84.2	9.1%

Group #4: Cytokines 2

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
AFP	1	48560.8	4731.4	9.7%
	2	17015.4	780.9	4.6%
ANGPTL4	1	1687.8	138.6	8.2%
	2	531.6	26.0	4.9%
CA125	1	32441.2	2890.1	8.9%
	2	11693.3	148.7	1.3%
FSH	1	23397.8	2698.1	11.5%
	2	10904.7	693.1	6.4%
hCGβ	1	158.5	18.7	11.8%
	2	67.8	7.3	10.7%
IGF-1R	1	33221.5	1086.1	3.3%
	2	11384.6	1078.3	9.5%
IL-1 RII	1	16723.0	1740.2	10.4%
	2	5451.8	270.8	5.0%
IL-18 Rβ	1	1003.2	54.9	5.5%
	2	322.8	12.9	4.0%
IL-21	1	16335.2	1945.4	11.9%
	2	5531.5	576.2	10.4%
IL-3	1	3126.1	68.6	2.2%
	2	1235.8	54.8	4.4%
Leptin	1	1680.6	41.4	2.5%
	2	609.6	74.7	12.3%
MMP-10	1	3340.2	156.7	4.7%
	2	1093.8	28.5	2.6%
MMP-13	1	1667.2	50.5	3.0%
	2	553.0	48.4	8.7%
MMP-3	1	50205.7	4303.1	8.6%
	2	16597.6	592.5	3.6%
MMP-8	1	50400.8	960.1	1.9%
	2	16379.1	1962.6	12.0%
NSE	1	59937.6	2553.8	4.3%
	2	20154.5	1494.5	7.4%
OSM	1	6391.3	531.6	8.3%
	2	2314.2	95.4	4.1%
Prolactin	1	40557.1	2577.1	6.4%
	2	16723.9	1220.4	7.3%
PSA-Free	1	66663.2	2146.1	3.2%
	2	22308.4	927.8	4.2%
Siglec-9	1	6774.6	861.3	12.7%
	2	2142.9	202.7	9.5%

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
TACE	1	66496.0	3454.1	5.2%
	2	22947.6	747.3	3.3%
TIMP-4	1	3332.0	148.2	4.4%
	2	1118.0	77.3	6.9%

Group #5: Receptors

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
TRAIL R3	1	33268.7	1405.0	4.2%
	2	11333.3	516.5	4.6%
DR6	1	28605.3	264.9	0.9%
	2	9225.2	275.2	3.0%
CD14	1	29472.0	769.5	2.6%
	2	10594.3	662.5	6.3%
CEACAM-1	1	33488.2	12.9	0.0%
	2	10539.0	338.2	3.2%
IL-1 R1	1	27218.4	646.0	2.4%
	2	8774.0	354.0	4.0%
PECAM-1	1	33706.7	7505.1	22.3%
	2	11039.4	1524.9	13.8%
E-Selectin	1	33427.2	122.0	0.4%
	2	11038.5	307.3	2.8%
CD105	1	10827.6	837.8	7.7%
	2	3882.2	294.0	7.6%
Trappin-2	1	11109.3	2111.6	19.0%
	2	3503.2	347.8	9.9%
ErbB3	1	26609.8	562.8	2.1%
	2	9015.2	269.0	3.0%
CD30	1	30981.0	3009.6	9.7%
	2	13756.0	2669.3	19.4%
IL-2 Ry	1	9957.5	135.3	1.4%
	2	3460.3	351.3	10.2%
CD80	1	15854.0	137.4	0.9%
	2	13174.6	935.6	7.1%
Contactin-2	1	33355.7	23.8	0.1%
	2	11052.4	68.0	0.6%
uPAR	1	33418.3	422.7	1.3%
	2	11012.6	719.9	6.5%
Dtk	1	33594.3	215.2	0.6%
	2	10894.5	362.4	3.3%
ALCAM	1	27459.3	6217.2	22.6%
	2	11037.3	596.7	5.4%
LIMP2	1	33197.9	616.2	1.9%
	2	11125.1	767.8	6.9%
MICA	1	30056.9	441.1	1.5%
	2	9948.0	504.3	5.1%
TIM-1	1	33747.1	4589.4	13.6%
	2	11243.7	921.8	8.2%

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
IL-17 RA	1	33252.0	1467.1	4.4%
	2	11102.8	957.5	8.6%
XEDAR	1	32224.5	5080.5	15.8%
	2	11718.3	872.3	7.4%
Fas	1	16737.7	64.9	0.4%
	2	5452.8	41.4	0.8%
IL-21 R	1	33380.9	99.9	0.3%
	2	10871.9	234.2	2.2%
CD40 ligand	1	3363.5	122.6	3.6%
	2	1179.7	101.2	8.6%
Flt-3 ligand	1	14119.5	1505.1	10.7%
	2	5575.4	209.7	3.8%
GITR	1	33194.5	186.2	0.6%
	2	11462.0	183.8	1.6%

Group #6: Growth Factors

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
GDF-15	1	2360.2	134.5	5.7%
	2	1251.2	90.9	7.3%
OPG	1	994.1	44.7	4.5%
	2	333.0	12.3	3.7%
VEGF-D	1	11712.3	2868.7	24.5%
	2	3592.4	172.0	4.8%
β -NGF	1	3322.2	1024.6	30.8%
	2	1155.6	64.8	5.6%
Growth Hormone	1	22079.4	752.3	3.4%
	2	9028.9	996.5	11.0%
IGF-1	1	50035.6	2752.4	5.5%
	2	16711.3	2011.5	12.0%
NGFR	1	33361.3	600.9	1.8%
	2	11064.8	238.2	2.2%
TGF- β 3	1	28064.1	2779.7	9.9%
	2	12958.4	1035.8	8.0%
BMP-4	1	6786.4	681.8	10.0%
	2	2160.0	195.5	9.1%
IGFBP-2	1	33247.8	1764.8	5.3%
	2	11278.9	1493.1	13.2%
BMP-7	1	33365.4	955.8	2.9%
	2	10581.8	817.2	7.7%
VEGF-A	1	1003.9	22.5	2.2%
	2	320.8	12.7	3.9%
bFGF	1	1000.2	79.2	7.9%
	2	325.3	9.2	2.8%
TGF β 1	1	66682.2	2996.6	4.5%
	2	22264.8	671.0	3.0%
SCF	1	8429.7	1440.8	17.1%
	2	2747.2	271.6	9.9%
IGFBP-6	1	33336.5	979.8	2.9%
	2	11067.4	1379.8	12.5%
VEGFR3	1	33366.5	452.1	1.4%
	2	11007.2	276.8	2.5%
VEGFR2	1	50188.8	2773.4	5.5%
	2	16169.3	344.5	2.1%
PlGF	1	3974.2	844.8	21.3%
	2	1748.7	382.5	21.9%
EG-VEGF	1	13383.1	1281.3	9.6%
	2	4415.3	259.5	5.9%

2) Inter-Assay Precision

Spiked standard protein in RayBio® Human Serum/Plasma Diluent (Item 10) was analyzed in triplicate across three independent assays. Samples were spiked in with the concentrations of protein standard 2 (Sample #1) and 3 (Sample #2). The inter-assay precision in CV of the samples is provided below.

Group #1: Inflammatory Cytokines

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
Eotaxin-2	1	1329.5	6.4	0.5%
	2	444.0	10.2	2.3%
BLC	1	499.3	0.1	0.0%
	2	169.4	0.1	0.1%
RANTES	1	502.2	0.9	0.2%
	2	161.8	4.1	2.5%
MCP-1	1	650.6	12.9	2.0%
	2	232.7	8.6	3.7%
IL-10	1	330.9	5.6	1.7%
	2	113.2	6.6	5.8%
PDGF-BB	1	479.5	7.5	1.6%
	2	183.5	14.5	7.9%
IFN- γ	1	196.3	1.8	0.9%
	2	69.2	3.3	4.7%
TNF- α	1	498.1	6.0	1.2%
	2	166.6	8.4	5.1%
IL-5	1	854.5	111.8	13.1%
	2	359.3	19.7	5.5%
IL-1 β	1	1984.8	21.5	1.1%
	2	677.7	12.9	1.9%
IL-6	1	2013.3	6.3	0.3%
	2	647.5	10.0	1.5%
IL-2	1	3353.9	61.7	1.8%
	2	1109.6	37.9	3.4%
IL-4	1	668.5	1.2	0.2%
	2	215.1	4.8	2.2%
G-CSF	1	3409.1	98.6	2.9%
	2	1101.6	23.4	2.1%
IL-12 p70	1	644.0	23.4	3.6%
	2	245.5	31.5	12.8%
IL-8	1	1347.8	18.8	1.4%
	2	435.6	12.5	2.9%
MIP-1 α	1	499.7	8.2	1.6%
	2	165.7	3.3	2.0%
IL-7	1	400.3	0.3	0.1%
	2	133.3	0.2	0.1%
IL-9	1	1639.3	18.3	1.1%
	2	560.2	21.8	3.9%
TIMP-2	1	32937.6	600.7	1.8%
	2	11371.0	302.0	2.7%
TNFR1	1	10258.9	738.7	7.2%
	2	3400.2	99.1	2.9%

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
IL-1Ra	1	9866.8	27.7	0.3%
	2	3437.5	32.4	0.9%
TNFR2	1	25855.6	1966.3	7.6%
	2	12303.1	2487.3	20.2%
IL-17	1	1676.9	7.4	0.4%
	2	537.6	15.1	2.8%
I-309	1	981.0	5.7	0.6%
	2	387.1	22.0	5.7%
IL-12 p40	1	6676.6	17.2	0.3%
	2	2194.1	55.9	2.5%
IL-13	1	333.0	1.1	0.3%
	2	110.7	2.9	2.6%
IL-15	1	10008.5	13.3	0.1%
	2	3305.6	44.9	1.4%
Eotaxin-1	1	500.4	0.6	0.1%
	2	164.7	3.2	2.0%
IL-16	1	10094.5	1484.4	14.7%
	2	3364.2	18.9	0.6%
MIP-1 δ	1	8709.4	490.2	5.6%
	2	3745.4	333.1	8.9%
TNF β	1	10038.7	64.0	0.6%
	2	3263.3	150.6	4.6%
IL-1 α	1	3364.6	41.0	1.2%
	2	1100.9	15.8	1.4%
ICAM-1	1	66636.8	65.0	0.1%
	2	22382.8	254.3	1.1%
IL-11	1	30648.2	3140.6	10.2%
	2	10753.7	291.1	2.7%
IFN α	1	24481.8	6313.4	25.8%
	2	11676.7	839.0	7.2%
M-CSF	1	3307.4	55.0	1.7%
	2	1133.4	30.2	2.7%
GM-CSF	1	9766.7	756.8	7.7%
	2	3313.8	37.4	1.1%
IL-28A	1	1663.8	2.8	0.2%
	2	547.5	11.6	2.1%
MIP-3 β	1	999.3	4.1	0.4%
	2	334.7	9.1	2.7%
MIG	1	979.0	6.7	0.7%
	2	366.4	16.2	4.4%

Group #2: Chemokines

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
TSLP	1	632.1	12.4	2.0%
	2	241.7	6.9	2.9%
Eotaxin-3	1	3337.1	2.1	0.1%
	2	1090.2	6.0	0.6%
MIP-1 β	1	3209.0	245.8	7.7%
	2	1120.9	14.7	1.3%
MDC	1	3338.8	34.1	1.0%
	2	1063.5	82.0	7.7%
I-TAC	1	1672.9	3.4	0.2%
	2	560.9	33.7	6.0%
TECK	1	994.7	10.0	1.0%
	2	345.1	32.6	9.4%
HCC-4	1	1628.7	18.4	1.1%
	2	595.8	46.2	7.8%
IL-18 Bpa	1	6435.6	398.9	6.2%
	2	2904.8	466.4	16.1%
NAP-2	1	47610.4	2883.0	6.1%
	2	23356.1	2064.4	8.8%
Osteopontin	1	66665.7	164.4	0.2%
	2	22234.5	594.1	2.7%
MIF	1	98.2	5.9	6.0%
	2	31.6	3.3	10.5%
LIF	1	33282.9	353.1	1.1%
	2	10486.1	664.7	6.3%
Axl	1	6622.7	42.1	0.6%
	2	2159.7	101.9	4.7%
Betacellulin	1	9952.6	1464.2	14.7%
	2	3292.9	61.2	1.9%
CXCL5	1	1920.3	148.5	7.7%
	2	679.7	16.4	2.4%
Lymphotactin	1	3469.7	288.8	8.3%
	2	1103.5	135.2	12.3%
SDF-1 α	1	663.5	4.7	0.7%
	2	224.2	8.4	3.7%
MPIF-1	1	2991.7	348.8	11.7%
	2	1029.8	77.8	7.6%
GRO α	1	992.9	31.5	3.2%
	2	326.0	7.7	2.3%
PARC	1	3340.9	520.8	15.6%
	2	1050.4	95.9	9.1%

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
IP-10	1	4919.6	164.3	3.3%
	2	1759.3	148.7	8.5%
MIP-3 α	1	1043.4	82.2	7.9%
	2	325.9	2.5	0.8%
TARC	1	934.8	125.0	13.4%
	2	362.8	43.1	11.9%
MCP-2	1	318.4	28.0	8.8%
	2	110.0	5.2	4.7%
MCP-3	1	165.7	1.1	0.7%
	2	56.9	2.0	3.6%
GCP-2	1	2058.3	145.8	7.1%
	2	670.0	36.0	5.4%
HCC-1	1	1995.9	9.8	0.5%
	2	616.0	74.5	12.1%
IL-31	1	31863.3	1039.0	3.3%
	2	11816.0	704.0	6.0%
IL-29	1	16412.5	353.9	2.2%
	2	5364.0	401.9	7.5%
IL-17F	1	1913.7	27.8	1.5%
	2	728.8	36.3	5.0%
MCP-4	1	987.8	10.1	1.0%
	2	365.8	19.7	5.4%
CCL28	1	980.0	13.7	1.4%
	2	360.3	28.1	7.8%
CTACK	1	6272.0	536.6	8.6%
	2	2230.4	139.4	6.3%

Group #3: Cytokines 1

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
AgRP	1	915.6	85.8	9.4%
	2	305.5	14.5	4.7%
Angiostatin	1	12505.1	803.8	6.4%
	2	4538.2	707.3	15.6%
LAP	1	4994.4	535.6	10.7%
	2	1707.4	366.7	21.5%
IL-2 R α	1	6703.2	20.1	0.3%
	2	2210.1	28.5	1.3%
Angiogenin	1	3178.9	60.6	1.9%
	2	1249.5	111.8	8.9%
PDGF-AB	1	331.4	2.6	0.8%
	2	113.3	2.4	2.1%
E-Cadherin	1	65353.4	426.3	0.7%
	2	22667.8	353.8	1.6%
Activin A	1	23864.3	2476.2	10.4%
	2	8825.5	122.2	1.4%
Cripto-1	1	7408.9	1572.1	21.2%
	2	2349.8	182.9	7.8%
ICAM-2	1	114966.6	3153.8	2.7%
	2	38988.9	3639.8	9.3%
IL-2 R β	1	92221.3	4161.6	4.5%
	2	40940.2	7098.8	17.3%
VEGFR1	1	25745.5	1232.2	4.8%
	2	9150.2	1091.0	11.9%
Follistatin	1	946.5	63.0	6.7%
	2	307.0	73.6	24.0%
Cathepsin S	1	28454.5	5430.5	19.1%
	2	8868.3	2461.7	27.8%
CD40	1	13349.8	1.6	0.0%
	2	4427.1	2.8	0.1%
Fc γ RIIB/C	1	45246.0	8423.6	18.6%
	2	25267.0	2441.5	9.7%
GP130	1	81479.9	11433.4	14.0%
	2	33868.1	7316.8	21.6%
Thrombopoietin	1	63582.6	355.6	0.6%
	2	29084.9	1335.9	4.6%
DAN	1	13400.2	108.7	0.8%
	2	4407.6	242.0	5.5%
Galectin-7	1	66634.3	9501.8	14.3%
	2	17453.9	3517.5	20.2%

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
Fas Ligand	1	331.6	2.5	0.8%
	2	111.7	4.2	3.8%
SDF-1 β	1	1001.5	1.5	0.1%
	2	327.0	6.1	1.9%
VEGF-C	1	11899.0	1376.5	11.6%
	2	5077.9	228.7	4.5%
TGF β 2	1	17972.7	3101.6	17.3%
	2	6212.8	382.8	6.2%
IL-13 R α 1	1	26883.0	231.3	0.9%
	2	8722.1	203.4	2.3%
IL-1 R4	1	1672.7	6.4	0.4%
	2	548.8	11.3	2.1%
Tie-2	1	26770.6	11.4	0.0%
	2	8632.5	44.7	0.5%
TSH	1	46710.9	1229.5	2.6%
	2	18541.2	628.1	3.4%
SHH-N	1	1821.6	540.7	29.7%
	2	540.6	143.0	26.5%
TREM-1	1	3242.1	76.6	2.4%
	2	1236.5	119.9	9.7%
IL-23	1	29.3	4.3	14.7%
	2	13.2	2.3	17.8%
TRAIL R4	1	2633.9	26.4	1.0%
	2	897.3	23.6	2.6%

Group #4: Cytokines 2

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
AFP	1	47435.4	5977.2	12.6%
	2	16150.2	919.9	5.7%
ANGPTL4	1	1677.8	9.9	0.6%
	2	540.7	8.4	1.6%
CA125	1	32596.5	293.9	0.9%
	2	11891.6	191.0	1.6%
FSH	1	39317.7	13804.9	35.1%
	2	14018.4	2989.7	21.3%
hCG β	1	160.1	3.3	2.1%
	2	64.5	6.1	9.4%
IGF-1R	1	33389.5	147.6	0.4%
	2	10986.0	365.1	3.3%
IL-1 RII	1	16730.6	54.6	0.3%
	2	5476.1	61.5	1.1%
IL-18 R β	1	1005.0	3.4	0.3%
	2	323.5	4.6	1.4%
IL-21	1	16453.5	238.3	1.4%
	2	5545.6	354.2	6.4%
IL-3	1	3146.2	94.1	3.0%
	2	1172.3	55.6	4.7%
Leptin	1	1683.0	7.1	0.4%
	2	539.7	61.2	11.3%
MMP-10	1	3336.0	6.8	0.2%
	2	1106.8	20.6	1.9%
MMP-13	1	1668.9	2.2	0.1%
	2	550.7	2.6	0.5%
MMP-3	1	51515.9	1818.6	3.5%
	2	16349.4	425.0	2.6%
MMP-8	1	50434.8	253.7	0.5%
	2	16497.6	132.8	0.8%
NSE	1	60023.9	116.5	0.2%
	2	20077.6	66.6	0.3%
OSM	1	6563.0	173.0	2.6%
	2	2195.0	166.5	7.6%
Prolactin	1	37923.3	13294.5	35.1%
	2	16923.8	192.7	1.1%
PSA-Free	1	66691.4	25.2	0.0%
	2	22278.8	63.0	0.3%
Siglec-9	1	6727.6	67.1	1.0%
	2	2171.9	76.2	3.5%

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
TACE	1	66583.9	743.6	1.1%
	2	22635.3	2287.2	10.1%
TIMP-4	1	3331.6	4.9	0.1%
	2	1121.2	21.5	1.9%

Group #5: Receptors

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
TRAIL R3	1	31709.7	4487.4	14.2%
	2	11924.5	719.3	6.0%
DR6	1	28328.2	1175.4	4.1%
	2	9527.9	810.9	8.5%
CD14	1	28585.7	1768.7	6.2%
	2	11348.8	1192.7	10.5%
CEACAM-1	1	33476.9	3026.2	9.0%
	2	12008.8	2115.7	17.6%
IL-1 R1	1	26472.5	3168.4	12.0%
	2	9110.4	1039.3	11.4%
PECAM-1	1	31662.2	4798.6	15.2%
	2	11915.0	565.6	4.7%
E-Selectin	1	33348.8	2306.5	6.9%
	2	11209.4	1469.9	13.1%
CD105	1	11004.7	454.8	4.1%
	2	3772.5	136.8	3.6%
Trappin-2	1	3144.6	797.8	25.4%
	2	3708.4	682.8	18.4%
ErbB3	1	26036.3	673.9	2.6%
	2	9289.1	203.6	2.2%
CD30	1	34245.0	11544.2	33.7%
	2	11235.1	1905.2	17.0%
IL-2 Ry	1	9802.0	265.7	2.7%
	2	3865.5	115.4	3.0%
CD80	1	15854.0	137.4	0.9%
	2	13174.6	935.6	7.1%
Contactin-2	1	33374.4	2552.6	7.6%
	2	11052.5	477.7	4.3%
uPAR	1	33138.4	2187.1	6.6%
	2	11442.8	706.9	6.2%
Dtk	1	33710.9	7511.2	22.3%
	2	11115.1	1212.9	10.9%
ALCAM	1	32925.5	7984.8	24.3%
	2	11712.6	687.2	5.9%
LIMP2	1	32489.1	1058.9	3.3%
	2	11982.2	955.1	8.0%
MICA	1	29608.7	1288.6	4.4%
	2	10462.2	960.9	9.2%
TIM-1	1	28630.8	2377.2	8.3%
	2	12236.2	1095.6	9.0%

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
IL-17 RA	1	32224.5	5080.5	15.8%
	2	11718.3	872.3	7.4%
XEDAR	1	46906.2	8641.2	18.4%
	2	20040.5	3058.0	15.3%
Fas	1	16676.8	1905.8	11.4%
	2	5498.2	270.3	4.9%
IL-21 R	1	33276.0	1892.6	5.7%
	2	11048.8	840.3	7.6%
CD40 ligand	1	3249.2	263.7	8.1%
	2	1296.6	239.5	18.5%
Flt-3 ligand	1	12381.8	895.4	7.2%
	2	5793.4	986.5	17.0%
GITR	1	33118.0	895.4	2.7%
	2	11568.9	986.5	8.5%

Group #6: Growth Factors

Target	Sample #	Mean (pg/mL)	Standard Deviation	CV %
GDF-15	1	2663.6	870.5	32.7%
	2	1312.6	55.6	4.2%
OPG	1	987.4	14.8	1.5%
	2	343.2	9.9	2.9%
VEGF-D	1	11861.6	491.2	4.1%
	2	3516.8	184.4	5.2%
β -NGF	1	2873.2	451.3	15.7%
	2	1095.8	271.0	24.7%
Growth Hormone	1	23204.2	4485.9	19.3%
	2	9017.0	533.8	5.9%
IGF-1	1	50208.3	394.0	0.8%
	2	16680.7	184.6	1.1%
NGFR	1	33224.8	336.5	1.0%
	2	11242.4	170.5	1.5%
TGF- β 3	1	23990.2	8912.8	37.2%
	2	10621.3	3847.3	36.2%
BMP-4	1	6175.2	648.3	10.5%
	2	2442.0	275.9	11.3%
IGFBP-2	1	33021.5	658.6	2.0%
	2	11569.9	580.3	5.0%
BMP-7	1	32620.4	1282.3	3.9%
	2	11801.6	1914.6	16.2%
VEGF-A	1	1005.3	4.6	0.5%
	2	321.1	12.1	3.8%
bFGF	1	324.3	3.6	1.1%
	2	147.8	3.6	2.4%
TGF β 1	1	66267.4	467.2	0.7%
	2	22345.5	223.7	1.0%
SCF	1	8428.5	188.7	2.2%
	2	2775.4	31.2	1.1%
IGFBP-6	1	33392.0	72.4	0.2%
	2	11050.7	241.4	2.2%
VEGFR3	1	33421.4	170.0	0.5%
	2	10838.9	509.7	4.7%
VEGFR2	1	50116.7	210.4	0.4%
	2	16318.6	777.1	4.8%
PlGF	1	4309.3	301.2	7.0%
	2	1576.8	190.3	12.1%
EG-VEGF	1	13392.0	17.7	0.1%
	2	4402.7	46.4	1.1%

NOTE: All data in Section VII were obtained using a Flat-Bottom plate. Five-parameter logistic curve fitting was used for generation of standard curve and calculation of concentrations.

VIII. Troubleshooting Guide

Issue	Possible Causes	Recommendations
Weak Signal	Low protein content in sample	Increase the length of the sample-bead incubation to overnight at 4°C (see Section VI.D “Assay Procedure”, step 3) Decrease sample dilution Note that even if the incubation is increased or the sample dilution is decreased, it is not guaranteed that the protein will be detected
	Sample matrix effect	Optimize sample dilution For serum or plasma samples, prepare the standards in the provided RayBio® Human Serum/Plasma Diluent (Item 10) Adjust the PE MFI using a small amount of beads from the negative control to around 10^{2-3}
	Improper storage of kit	Store kit at suggested temperature and use within 6 months of receipt
Poor Standard Curve	Cross-contamination from neighboring wells	Avoid overflowing wells during wash steps
	Lyophilized Protein Standard Mix (Items 11-16) not properly prepared	Serially dilute the Lyophilized Protein Standard Mix (Items 11-16) according to this manual
	Lyophilized Protein Standard Mix (Items 11-16) degraded or not properly diluted	Store Lyophilized Protein Standard at -20°C or lower. Reconstitute the Lyophilized Protein Standard Mix (Items 11-16) on ice before making serial dilutions
High Background	Improper flow cytometer setup and optimization	Run Setup Bead Cocktail (Item 24) before assay. Make sure the top standard signal is not out of the linear range
	PE voltage PMT voltage is not set up correctly	Set PE voltage using a small amount of beads from the negative control so that the PE MFI is around 10^{2-3}
	Insufficient washing	Increase wash time Use more wash buffer
Bead Loss	Magnetic separator unit not strong enough to hold beads	Try a different magnetic separator unit or leave the 96-well plate with beads on the unit for longer before washing and in-between dispensing/aspirating wash buffer
	Aspiration/Dispensing not optimized	Edit the rate of aspiration and/or dispensing wash buffer using the plate washing machine's programming
	Washing system is not compatible with magnetic beads	Switch to using a Filter Plate to avoid any bead loss

IX. Appendix

Data Analysis using Batch Analysis

Batch analysis allows exporting PE MFI data of each bead population directly from FACS Diva software. Before batch analysis, it is important to create statistics view from the dot plot of gated beads population and choose “PE-A Median” to be shown (Figure 3D-E). Batch analysis can be done in either manual tube mode or HTS mode. Both methods for batch analysis are described below (Figure 4 and 5).

1. Create a dot plot to show all beads populations and make a gate for each population (see Figure 3 in Section VI.E). Right click on the dot plot to “Create Statistics View”, then edit the statistics view so that “PE-A Median” of gated beads populations are shown (Figure 3E).
2. **Batch Analysis from Manual Tube Mode:** Right click on the open folder containing your experiment data and select ‘Batch Analysis.’ When the pop-up appears, select the desired destination folder, and then hit start.
3. **Batch Analysis from HTS Mode:** When the HTS is done running samples, click on the ‘Analysis’ tab and highlight all the samples. Right click and select ‘Batch Analysis.’ After selecting Batch Analysis through the HTS mode, the steps from Manual mode are the same.
4. Open the CSV file in the destination folder and select ‘Save As’ to save the data as an Excel workbook (Figure 6).

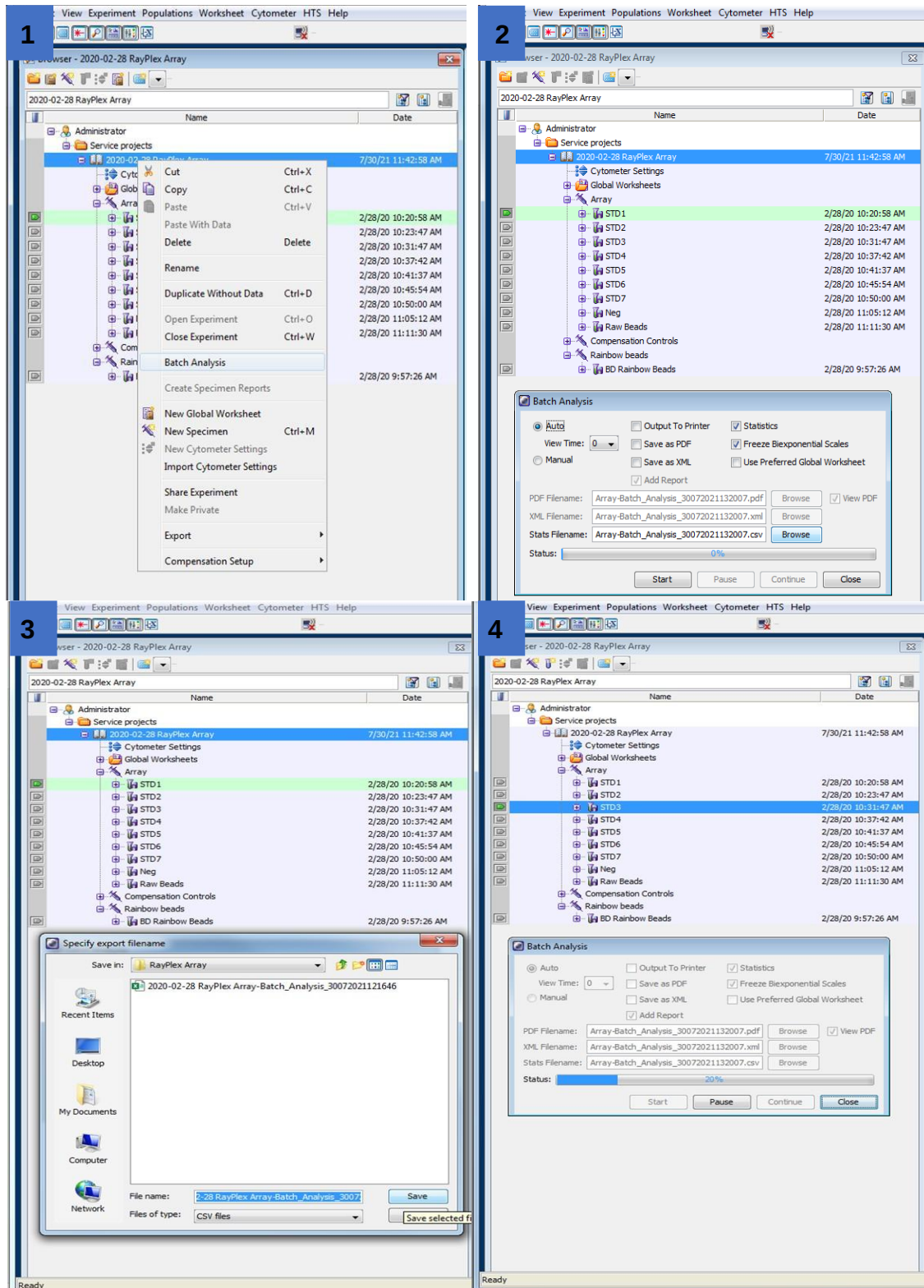


Figure 4. Screen shot showing how to begin the batch analysis through manual tube mode.

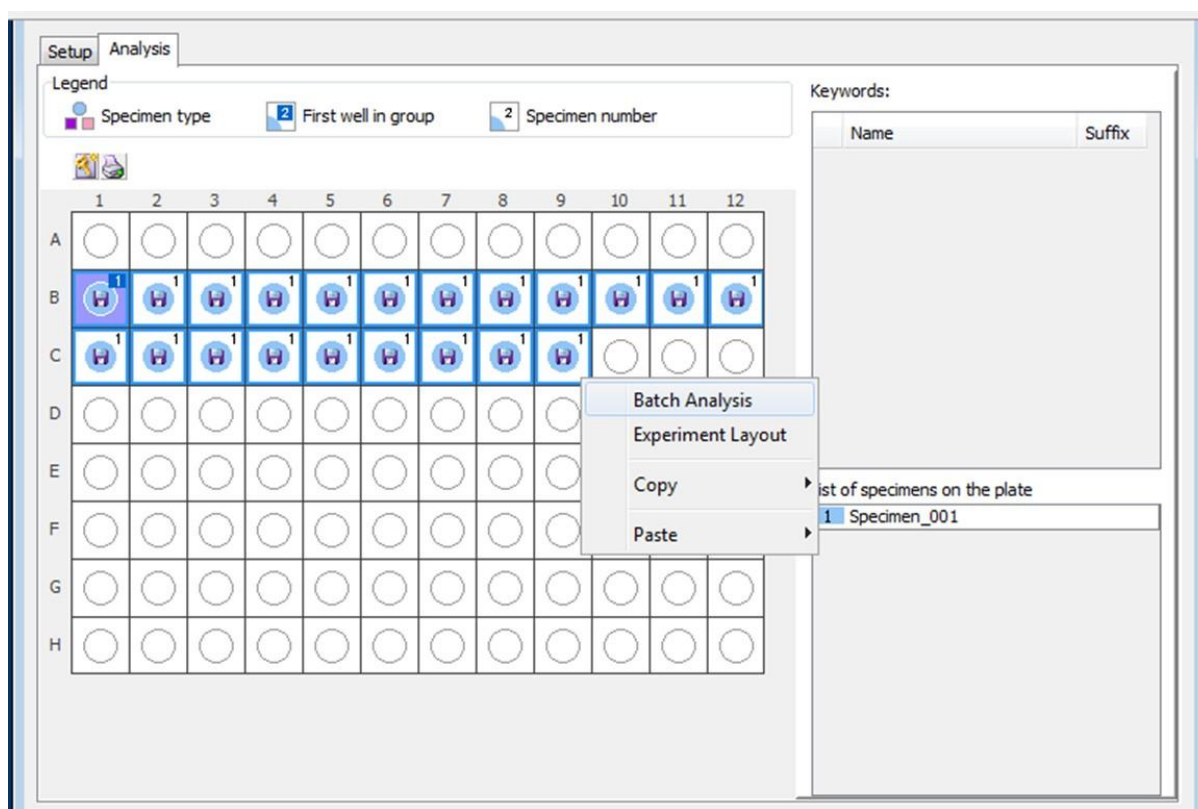


Figure 5. Screen shot showing how to begin HTS batch analysis.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Tube Name	R1 PE-A Median	R2 PE-A Median	R4 PE-A Median	R5 PE-A Median	R6 PE-A Median	R7 PE-A Median	R8 PE-A Median	R9 PE-A Median	R10 PE-A Median	R12 PE-A Median	R13 PE-A Median	R14 PE-A Median	
2	STD1	42203	35160	38647	48464	34527	28324	17515	15124	16826	14729	15436	16014	
3	STD2	24909	20453	22914	27861	20279	15538	10087	8766	10267	8787	9270	9982	
4	STD3	14359	11430	12524	16493	11076	7942	5942	4757	5882	5029	5248	5735	
5	STD4	7268	5314	5760	8796	5327	3730	3017	2098	2580	2201	2248	2607	
6	STD5	4158	2565	2781	4726	2554	1904	1461	963	1230	1027	1035	1226	
7	STD6	2639	1333	1437	3354	1327	1074	712	503	612	522	532	646	
8	STD7	1928	749	823	2339	835	682	393	286	360	305	318	392	
9	Neg	457	378	430	1676	456	377	182	134	174	140	143	206	
10	Raw Beads	4	2	2	6	15	43	1	0	1	2	6	10	
11														
12														
13														
14														
15														

Figure 6. Representative raw data obtained from batch analysis. Rows = data from samples. Left-most column = standard or sample name. (The names of beads are variable for different assays.

X. Notes

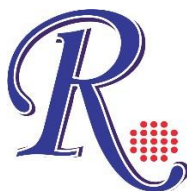
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