bio-techne

R&D SYSTEMS

Luminex® Performance Assay

NHP XL Cytokine Base Kit

Catalog Number LNHPXL000

For the simultaneous quantitative determination of multiple non-human primate biomarker concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Cytokines are intercellular signaling proteins released from a wide variety of cells and tissues. They play an integral role in regulating growth and cellular proliferation as well as modulating host response to infection, injury, and inflammation. Cytokines also influence reproduction and bone remodeling. A large number of cytokines are pleiotropic and share similar functions. In addition, many cytokines influence the production of other cytokines. Analysis and quantification of cytokines in biological fluids has thus become increasingly important. Methods such as bioassay, enzyme-linked immunosorbent assay (ELISA), intracellular staining, ribonuclease protection assay (RPA) and polymerase chain reaction (PCR) have all been used for quantifying cytokines, however, each of these techniques has limitations associated with it. These techniques are not capable of measuring multiple cytokines simultaneously in a limited sample volume.

Assessing the levels of multiple cytokines may be more revealing than analyzing a single protein. Quantifying multiple cytokines on an individual level can be time consuming and expensive. When combined with separately available analyte-specific microparticle sets, this kit is an excellent tool for simultaneously assessing the levels of multiple non-human primate cytokines in a single sample. Any combination of the following microparticle sets are suitable for use with this base kit.

Analyte	Catalog #	Microparticle Region
BDNF	LNHPXL848	12
CCL2/MCP-1	LNHPXL279	15
CCL4/MIP-1β	LNHPXL271	19
CCL5/RANTES	LNHPXL678	57
CCL11/Eotaxin	LNHPXL320	14
CCL20/MIP-3α	LNHPXL360	21
CD40 Ligand	LNHPXL617	20
CXCL2/GROβ	LNHPXL276	56
CXCL10/IP-10	LNHPXL266	22
CXCL11/I-TAC	LNHPXL672	25
CXCL13/BLC/BCA-1	LNHPXL801	13
FGF basic	LNHPXL233	26
G-CSF	LNHPXL214	27
GM-CSF	LNHPXL615	28
Granzyme B	LNHPXL2906	30
IFN-α	LNHPXL9345	33
IFN-β	LNHPXL814	29
IFN-γ	LNHPXL285	34
IL-1β	LNHPXL201	35

Analyte	Catalog #	Microparticle Region
IL-10	LNHPXL217	37
IL-12 p70	LNHPXL219	38
IL-13	LNHPXL213	39
IL-15	LNHPXL247	42
IL-17A	LNHPXL317	43
IL-2	LNHPXL202	45
IL-21	LNHPXL8879	46
IL-4	LNHPXL204	47
IL-5	LNHPXL405	44
IL-6	LNHPXL206	51
IL-7	LNHPXL207	52
IL-8/CXCL8	LNHPXL208	53
PDGF-AA	LNHPXL221	54
PDGF-BB	LNHPXL220	55
PD-L1	LNHPXL156	48
TGF-α	LNHPXL239	61
TNF-α	LNHPXL10702	62
VEGF	LNHPXL293	63

PRINCIPLE OF THE ASSAY

Luminex® Performance Assay multiplex kits are designed for use with any Luminex analyzer including the MAGPIX®, Luminex® 100/200™, FLEXMAP 3D®, xMAP INTELLIFLEX®, or Bio-Rad® Bio-Plex®, dual laser, flow-based sorting and detection platforms.

Analyte-specific antibodies are pre-coated onto magnetic microparticles embedded with fluorophores at set ratios for each unique microparticle region. Microparticles, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the biotinylated antibody, is added to each well. Final washes remove unbound Streptavidin-PE, the microparticles are resuspended in buffer and read using the MAGPIX®. A magnet in the analyzer captures and holds the superparamagnetic microparticles in a monolayer. Two spectrally distinct Light Emitting Diodes (LEDs) illuminate the microparticles. One LED excites the dyes inside each microparticle to identify the region and the second LED excites the PE to measure the amount of analyte bound to the microparticle. A sample from each well is imaged with a CCD camera with a set of filters to differentiate excitation levels.

Analysis with the Luminex® 100/200™, FLEXMAP 3D®, xMAP INTELLIFLEX®, or Bio-Rad® Bio-Plex® uses one laser to excite the dyes inside each microparticle to identify the microparticle region and the second laser to excite the PE to measure the amount of analyte bound to the microparticle.All excitation emitted as each microparticle passes through the flow cell is then analyzed to differentiate excitation levels using a Photomultiplier Tube (PMT) and an Avalanche Photodiode.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until these factors have been tested in the Luminex® Performance Assay, the possibility of interference cannot be excluded.
- Luminex® Performance Assays afford the user the benefit of multi-analyte analysis of biomarkers in a single complex sample. For each sample type, a single multipurpose diluent is used to optimize recovery, linearity, and reproducibility. Such a multipurpose diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions, therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.
- **Only the analytes listed on the Standard Value Card can be measured with this kit.**

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past the kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED, DILUTED, OR RECONSTITUTED MATERIAL
NHP XL Panel Standard Cocktail 1	899321	2 vials of recombinant biomarkers in a buffered protein base with preservatives; lyophilized.	Discard after use. Use a fresh standard and control for each assay.
NHP XL Panel Standard Cocktail 2	899322	2 vials of recombinant biomarkers in a buffered protein base with blue dye and preservatives; lyophilized.	
NHP XL Panel High Control	899326	2 vials of recombinant biomarkers in a buffered protein base with preservatives; lyophilized.	
NHP XL Panel Low Control	899325	2 vials of recombinant biomarkers in a buffered protein base with preservatives; lyophilized.	
NHP XL Panel Biotin-Antibody Cocktail	899323	1 vial of a concentrated biotinylated antibody cocktail; lyophilized.	May be stored for up to 1 month at 2-8 °C.* *Prepare fresh 1X solutions at the time of assay. Discard after use.*
Streptavidin-PE	893535	0.250 mL of a concentrated streptavidin-phycoerythrin conjugate with preservatives.	
Assay Diluent RD2-1	895970	2 vials (11 mL/vial) of a buffered protein base with preservative.	May be stored for up to 1 month at 2-8 °C.*
Calibrator Diluent RD6-65	895098	21 mL of a buffered protein base with preservatives. *Use diluted 1:2 for cell culture supernate samples. Use undiluted for serum and plasma samples.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Microplate	641385	1 flat-bottomed 96-well microplate used as a vessel for the assay.	
Mixing Bottles	895505	2 empty 8 mL bottles used for mixing microparticles with RD2-1.	
Plate Sealers	640445	4 adhesive foil strips.	
Control Mean Value Card	700152	1 card listing the low and high mean control values. For cell culture supernate assay.	
Control Mean Value Card	700153	1 card listing the low and high mean control values. For serum and plasma assay.	
Standard Value Card	700154	1 card listing the standard reconstitution volume and working standard concentrations for this lot of base kit.	

*Provided this is within the expiration date of the kit.

Additional wash buffer (#WA126) and plates (#LYX010) are available for purchase.

OTHER SUPPLIES REQUIRED

- **Luminex® Performance Assay analyte-specific kit(s) (see page 1)**
- MAGPIX®, Luminex® 100/200™, FLEXMAP 3D®, xMAP INTELLIFLEX®, or Bio-Rad® Bio-Plex® analyzer with X-Y platform
- Hand-held microplate magnet or platewasher with a magnetic platform
- Pipettes and pipette tips
- Deionized or distilled water
- Multi-channel pipette, manifold dispenser, or automated dispensing unit
- 50 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 800 ± 50 rpm
- Microcentrifuge
- **Polypropylene** test tubes for dilution of standards and samples

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Protect microparticles and Streptavidin-PE from light at all times to prevent photobleaching.

PRECAUTIONS

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifuging for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma and platelet-poor plasma are not validated for use in this assay. Hemolyzed, icteric, and lipemic samples are not suitable for use in this assay. Serum, Heparin plasma, and EDTA plasma samples are not suitable for use in the FGF basic and TGF-α assays. Heparin plasma, EDTA plasma, and cell culture supernate samples are not suitable for use in the CCL2/MCP-1 assay.*

SAMPLE PREPARATION

Use polypropylene tubes.

Note: *On the day of the assay, ALL fresh and previously frozen serum and plasma samples require centrifugation at 16,000 x g for 4 minutes immediately prior to use or dilution.*

Cell culture supernate samples require a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD6-65 (diluted 1:2)*. Mix thoroughly.

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD6-65. Mix thoroughly.

When assaying BDNF, IL-8/CXCL8, PDGF-BB, CXCL2/GROβ, and CCL5/RANTES serum samples must be further diluted 5-fold to a final 10-fold dilution. A suggested 10-fold dilution is 30 µL of the 2-fold diluted sample + 120 µL of Calibrator Diluent RD6-65. Mix thoroughly.

When assaying G-CSF, BDNF, and IL-8/CXCL8 plasma samples must be further diluted 5-fold to a final 10-fold dilution. A suggested 10-fold dilution is 30 µL of the 2-fold diluted sample + 120 µL of Calibrator Diluent RD6-65. Mix thoroughly.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

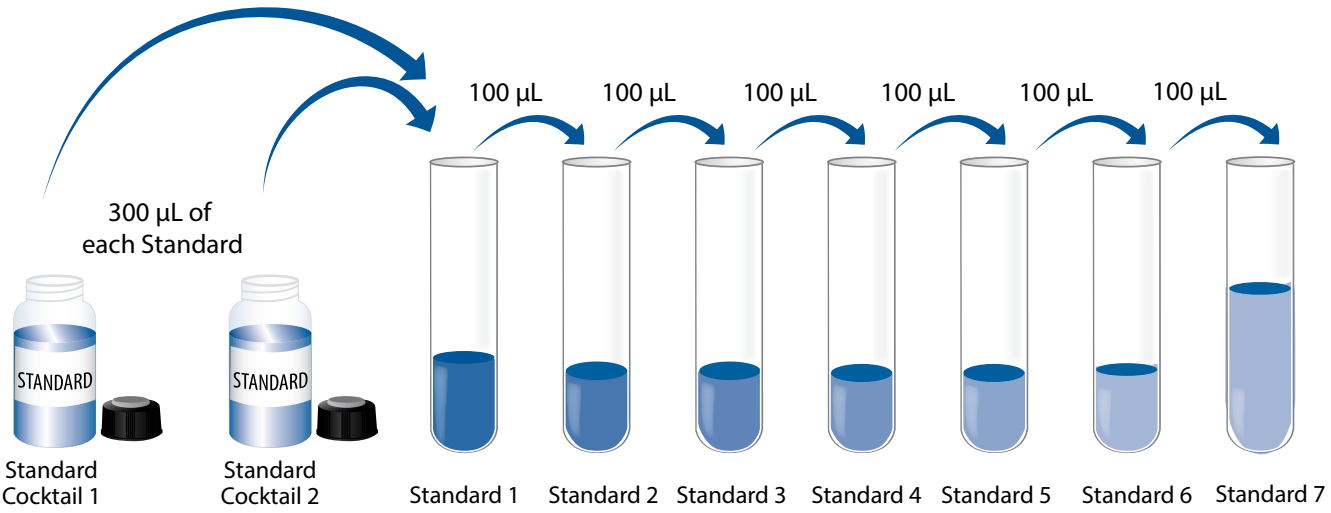
Calibrator Diluent RD6-65 (diluted 1:2) - **For cell culture supernate samples only.** Add 10 mL of Calibrator Diluent RD6-65 to 10 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD6-65 (diluted 1:2).

Low and High Controls - **Refer to the vial label for reconstitute volume.** Reconstitute the low and high controls with Calibrator Diluent RD6-65 (diluted 1:2) (*for cell culture supernate samples*) or Calibrator Diluent RD6-65 (*for serum/plasma samples*). Allow controls to sit for a minimum of 15 minutes with gentle agitation. This produces a 1X stock of each control. Discard low and high controls after use.

Biotin-Antibody Cocktail - **Refer to the vial label for reconstitution volume.** Reconstitute the NHP XL Panel Biotin-Antibody Cocktail with Assay Diluent RD2-1. Allow the antibody cocktail to sit for a minimum of 20 minutes or a minimum of 5 minutes on a nutator.

Standard - **Refer to the Standard Value Card for the reconstitution volume and assigned values.** Reconstitute Standard Cocktail 1 and Standard Cocktail 2 with Calibrator Diluent RD6-65 (diluted 1:2) (*for cell culture supernate samples*) or Calibrator RD6-65 (*for serum/plasma samples*). Allow the standard to sit for a minimum of 15 minutes prior to making dilutions. This produces a 2X stock of each standard cocktail.
Note: *Do not vortex standard cocktail. Gentle agitation should be initiated only after the 15-minute reconstitution step is complete.*

Use polypropylene tubes. Pipette 300 µL of reconstituted Standard Cocktail 1 and 300 µL of reconstituted Standard Cocktail 2 into the Standard 1 tube. Pipette 200 µL of Calibrator Diluent RD6-65 (diluted 1:2) (*for cell culture supernate samples*) or Calibrator RD6-65 (*for serum/plasma samples*) into the remaining tubes. Use Standard 1 to produce a 3-fold dilution series (below). Mix each tube thoroughly before the next transfer. Standard 1 serves as the high standard. The appropriate calibrator diluent serves as the blank.



DILUTED MICROPARTICLE COCKTAIL PREPARATION

1. Centrifuge each Microparticle Concentrate vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vials to resuspend the microparticles, taking precautions not to invert the vials.
3. Dilute the Microparticle Concentrates using Diluent RD2-1 in the mixing bottle provided. The volume of the Microparticle Concentrate listed in the table below is for each analyte (*e.g.* if measuring a full plate of IL-6 and IL-8, add 50 µL of IL-6 Microparticle Concentrate and 50 µL of IL-8 Microparticle Concentrate to 5 mL of Assay Diluent RD2-1.

Number of Wells Used	Microparticle Concentrate	+	Assay Diluent RD2-1
96	50.0 µL	+	5.00 mL
72	37.5 µL	+	3.75 mL
48	25.0 µL	+	2.50 mL
24	12.5 µL	+	1.25 mL

Note: *Protect microparticles from light during handling. Diluted microparticles cannot be stored. Prepare microparticles within 30 minutes of use.*

DILUTED BIOTIN-ANTIBODY COCKTAIL PREPARATION

1. Dilute the reconstituted NHP XL Panel Biotin-Antibody Cocktail in Assay Diluent RD2-1. Mix gently.

Number of Wells Used	Biotin-Antibody Cocktail	+	Assay Diluent RD2-1
96	0.500 mL	+	5.00 mL
72	0.375 mL	+	3.75 mL
48	0.250 mL	+	2.50 mL
24	0.125 mL	+	1.25 mL

STREPTAVIDIN-PE PREPARATION

Use a polypropylene amber bottle or a polypropylene tube wrapped with aluminum foil. Protect Streptavidin-PE from light during handling and storage.

1. Centrifuge the Streptavidin-PE vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Streptavidin-PE concentrate in Wash Buffer.

Number of Wells Used	Streptavidin-PE Concentrate	+	Wash Buffer
96	220 µL	+	5.35 mL
72	165 µL	+	4.00 mL
48	110 µL	+	2.65 mL
24	55 µL	+	1.35 mL

INSTRUMENT SETTINGS

Note: *Adjust the probe height setting on the analyzer to avoid puncturing the plate. Calibrate the analyzer using the proper reagents for superparamagnetic microparticles (refer to instrument manual).*

MAGPIX® analyzer:

a) Sample volume:

i. 1-25 analytes: 50 µL

ii. > 25 analytes: 35 µL

b) Assign the microparticle region for each analyte being measured (see page 1)

c) 50 count/region

d) Collect Median Fluorescence Intensity (MFI)

Luminex® 100/200™, FLEXMAP 3D®, xMAP INTELLIFLEX®, and Bio-Rad® Bio-Plex® analyzers:

Note: *Ensure that the instrument flow rate is set to the default of 60 µL/minute (fast) for all flow based analyzers.*

a) Sample volume: 50 µL

b) Bead Type:

i. Luminex® 100/200™ , FLEXMAP 3D®, and xMAP INTELLIFLEX® select MagPlex

ii. Bio-Rad® Bio-Plex® Manager use Bio-Plex® MagPlex Beads (Magnetic)

c) Doublet Discriminator gates:

i. Luminex® 100/200™ and FLEXMAP 3D®, set at 8000 and 16,500

ii. xMAP INTELLIFLEX® set at 7000 and 17,000

iii. Bio-Rad® Bio-Plex® Manager set at 8000 and 23,000

d) Reporter Gain Setting:

i. Luminex® 100/200™ use Default setting

ii. FLEXMAP 3D® use Standard PMT setting

iii. xMAP INTELLIFLEX® use Luminex® 200™ Operating Mode on Low PMT setting

iv. Bio-Rad® Bio-Plex® Manager use the low RP1 target value for the CAL2 setting

e) Assign the microparticle region for each analyte being measured (see page 1)

f) 50 count/region

g) Collect MFI

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.

Note: *Protect microparticles and Streptavidin-PE from light at all times.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 50 µL of standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
3. Resuspend the diluted Microparticle Cocktail by inversion or vortexing. Add 50 µL of the microparticle cocktail to each well of the microplate. Securely cover with a foil plate sealer. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 800 ± 50 rpm.
4. Using a magnetic device designed to accommodate a microplate, wash by applying the magnet to the bottom of the microplate, allow 1 minute before removing the liquid, filling each well with Wash Buffer (100 µL) and allow 1 minute before removing the liquid again. Uniform removal of liquid is essential for good performance. **Note:** ***Do NOT blot; this may cause a loss of microparticles.*** Perform the wash procedure three times.
5. Add 50 µL of diluted Biotin-Antibody Cocktail to all wells. Securely cover with a foil plate sealer and incubate for 1 hour at room temperature on the shaker set at 800 ± 50 rpm.
6. Repeat the wash as in step 4.
7. Add 50 µL of diluted Streptavidin-PE to all wells. Securely cover with a foil plate sealer and incubate for 30 minutes at room temperature on the shaker set at 800 ± 50 rpm.
8. Repeat the wash as in step 4.
9. Resuspend the microparticles by adding 100 µL of Wash Buffer to each well. Incubate for 2 minutes on the shaker set at 800 ± 50 rpm.
10. Read within 90 minutes using the Luminex® or Bio-Rad® analyzer.
    Note: *Resuspend microparticles immediately prior to reading by shaking the plate for 2 minutes on the plate shaker set at 800* ± 50 rpm.

*Samples require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

Note: *Protect microparticles and Streptavidin-PE from light at all times.*

❶ Prepare all reagents as instructed.

↓

❷ Add 50 µL of standard, control, or sample* to each well.

↓

❸ Add 50 µL of diluted Microparticle Cocktail to each well.
Incubate for 2 hours at RT on a shaker at 800 rpm.

↓

❹ Wash by removing the liquid from each well, filling with 100 µL Wash Buffer, and removing the liquid again.
Perform the wash 3 times.

↓

❺ Add 50 µL of diluted Biotin-Antibody Cocktail to each well.
Cover and incubate for 1 hour at RT on the shaker at 800 rpm.

↓

❻ Repeat the wash as in step 4.

↓

❼ Add 50 µL of diluted Streptavidin-PE to each well.
Incubate for 30 minutes at RT on the shaker at 800 rpm.

↓

❽ Repeat the wash as in step 4.

↓

❾ Add 100 µL of Wash Buffer to each well.
Incubate for 2 minutes at RT on the shaker at 800 rpm.

↓

❿ Read within 90 minutes using a Luminex® or Bio-Rad® analyzer
Note: *Resuspend microparticles immediately prior to reading.*

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Use the Standard concentrations on the Standard Value Card as the high standard concentrations. Calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard or sample and subtract the average blank Median Fluorescence Intensity (MFI).

Create a standard curve for each analyte by reducing the data using computer software, such as Bio-Techne® Quantist™, capable of generating a five parameter logistic (5-PL) curve-fit.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This assay is calibrated against highly purified recombinant proteins produced at R&D Systems®.

PRECISION

Intra-Assay Precision - Generated form the mean of the %CV's from 40 reportable results across two different concentrations of analytes in a single serum assay.

Inter-Assay Precision - Generated form the mean of the %CV's across two different concentrations of analytes across 31 different serum assays.

Analyte	Intra-Assay (%CV)	Inter-Assay (%CV)
BDNF	8.16	13.3
CCL2/MCP-1	3.02	10.5
CCL4/MIP-1β	5.63	10.5
CCL5/RANTES	3.72	17.0
CCL11/Eotaxin	7.98	15.4
CCL20/MIP-3α	8.41	17.3
CD40 Ligand	9.30	15.0
CXCL2/GROβ	7.76	13.1
CXCL10/IP-10	2.95	12.2
CXCL11/I-TAC	6.23	13.7
CXCL13/BLC	5.79	12.5
FGF basic	5.60	13.1
G-CSF	5.55	14.2
GM-CSF	7.11	14.1
Granzyme B	9.76	18.6
IFN-α	5.17	12.4
IFN-β	10.9	15.2
IFN-γ	6.36	13.0
IL-1β	2.55	12.7

Analyte	Intra-Assay (%CV)	Inter-Assay (%CV)
IL-10	8.55	14.1
IL-12 p70	4.92	17.7
IL-13	7.97	17.5
IL-15	4.99	18.2
IL-17A	4.38	19.0
IL-2	5.32	18.1
IL-21	5.84	19.0
IL-4	4.92	17.5
IL-5	4.14	16.5
IL-6	6.80	17.8
IL-7	6.57	17.7
IL-8/CXCL8	6.87	17.5
PDGF-AA	6.52	25.0
PDGF-BB	3.37	16.9
PD-L1/B7-H1	8.36	19.4
TGF-α	5.43	18.7
TNF-α	3.68	17.2
VEGF	4.79	18.4

ACCURACY

Natural Linearity - The data represents mean linearity in serum matrix samples (n=8).

Spike Recovery - The data represents mean percent recovery of spiked standards ranging from low, medium, and high concentration in serum matrix samples (n=8).

Analyte	Natural Linearity Average %	Spiked Recovery Average %
BDNF	114	HE
CCL2/MCP-1	75	135
CCL4/MIP-1β	NO	90
CCL5/RANTES	98	HE
CCL11/Eotaxin	93	138
CCL20/MIP-3α	105	111
CD40 Ligand	73	105
CXCL2/GROβ	95	81
CXCL10/IP-10	78	72
CXCL11/I-TAC	NO	97
CXCL13/BLC	NO	114
FGF basic	NV	
G-CSF	NO	67
GM-CSF	NO	111
Granzyme B	NO	122
IFN-α	NO	118
IFN-β	NO	71
IFN-γ	NO	124
IL-1β	NO	68

Analyte	Natural Linearity Average %	Spiked Recovery Average %
IL-10	NO	104
IL-12 p70	NO	109
IL-13	NO	118
IL-15	NO	90
IL-17A	NO	90
IL-2	NO	89
IL-21	NO	121
IL-4	NO	80
IL-5	NO	95
IL-6	NO	84
IL-7	NO	97
IL-8/CXCL8	121	HE
PDGF-AA	101	99
PDGF-BB	103	HE
PD-L1/B7-H1	80	HE
TGF-α	NV	
TNF-α	NO	85
VEGF	NO	97

" NO " Natural linearity was not observed.

" HE " Spiked recovery was not observed due to natural linearity in high endogenous samples.

"NV" Analyte is not validated for serum assay.

SPECIFICITY

The assay was tested for cross-reactivity and interference with the following factors. Less than 1.0% cross-reactivity and interference was observed unless otherwise noted.

Recombinant VEGF/PlGF cross-reacts at approximately 12.2% in the assay.

Recombinant IL-17A/F cross-reacts with IL-17A at approximately 11.6% in the assay.

Recombinant PlGF interferes with VEGF at concentrations ≥ 1000 pg/mL.

Recombinant VEGF R1 interferes with VEGF at concentrations ≥ 2000 pg/mL.

Recombinant antigen:

4-1BB Ligand
6Ckine
α_2-Macroglobulin
Amphiregulin
Angiogenin
APRIL
B7-1
B7-2
B7-H2
B7-H3
B7-H4
B7-H6
B7-H7
BAFF/BLyS
BLC/BCA-1
Cathepsin C
Cathepsin H
CCL7/MCP-3
CCL22/MDC
CD27 Ligand/CD70
CD30 Ligand
CNTF
Cripto-1
CXCL5/ENA-78
CXCL6/GCP-2/LIX
CXCL7/NAP-2
EGFR
ErbB2
ErbB3
ErbB4
Fas Ligand
FGF R1α (IIIb)
FGF R1α (IIIc)
FGF Rβ (IIIb)
FGF R1β (IIIc)
FGF acidic
FGF-4
FGF-5
FGF-6
FGF-7/KGF
FGF-16
Fibrinogen
G-CSF R
GITR Ligand
GM-CSF Rα
GM-CSF Rβ
gp130
GROγ
HB-EGF
HCC-4
HGF
I-309
IFN-α/β R1
IFN-α/β R2
IFN-γ R1
IGF-I
IGF-II
IL-1 RAcP/IL-1 R3
IL-1 RI
IL-1 RII
IL-1 Rα
IL-2 Rα
IL-3 Rα
IL-4 R
IL-5 Rα
IL-6 Rα/gp130
IL-9
IL-11
IL-12 Rβ1
IL-12 Rβ2
IL-12p40
IL-13 Rα1
IL-13 Rα2
IL-15 Rα
IL-17 R
IL-17 RC
IL-17 RD
IL-17B
IL-17B R
IL-17C
IL-17D
IL-17F
IL-18
IL-18 Rα
IL-18 Rβ
IL-23p19/IL-12 p40
IL-27
IL-36Ra/FIL-1δ
IL-36α
IL-36β
IL-36γ
IL-37/FIL-1ζ/IL-1F7
IL-38/IL-1F10
Integrin α5β3
I-TAC
LAP (TGF-β1)
LIF
LIF R
LIGHT
LT-α/TNF-β
LT α1/β2
LT α2/β1
MCP-2
MCP-4
M-CSF
MFG-E8
MIG
OPG
OSM
OX40 Ligand
PARC/MIP-4
PD-1
PD-L2
PF-4
Pleiotrophin/PTN
SDF-1α
SDF-1β
Serpin B9/PI-9
ST2
Syndecan-2
THBS
TNF RI
TNF RII
TRAIL R3
TRAIL R4
TRANCE
TSG-6
TGS-14/Pentraxin-3
TWEAK
VEGF R1/Flt-1
VEGF R2/Flk-1/KDR

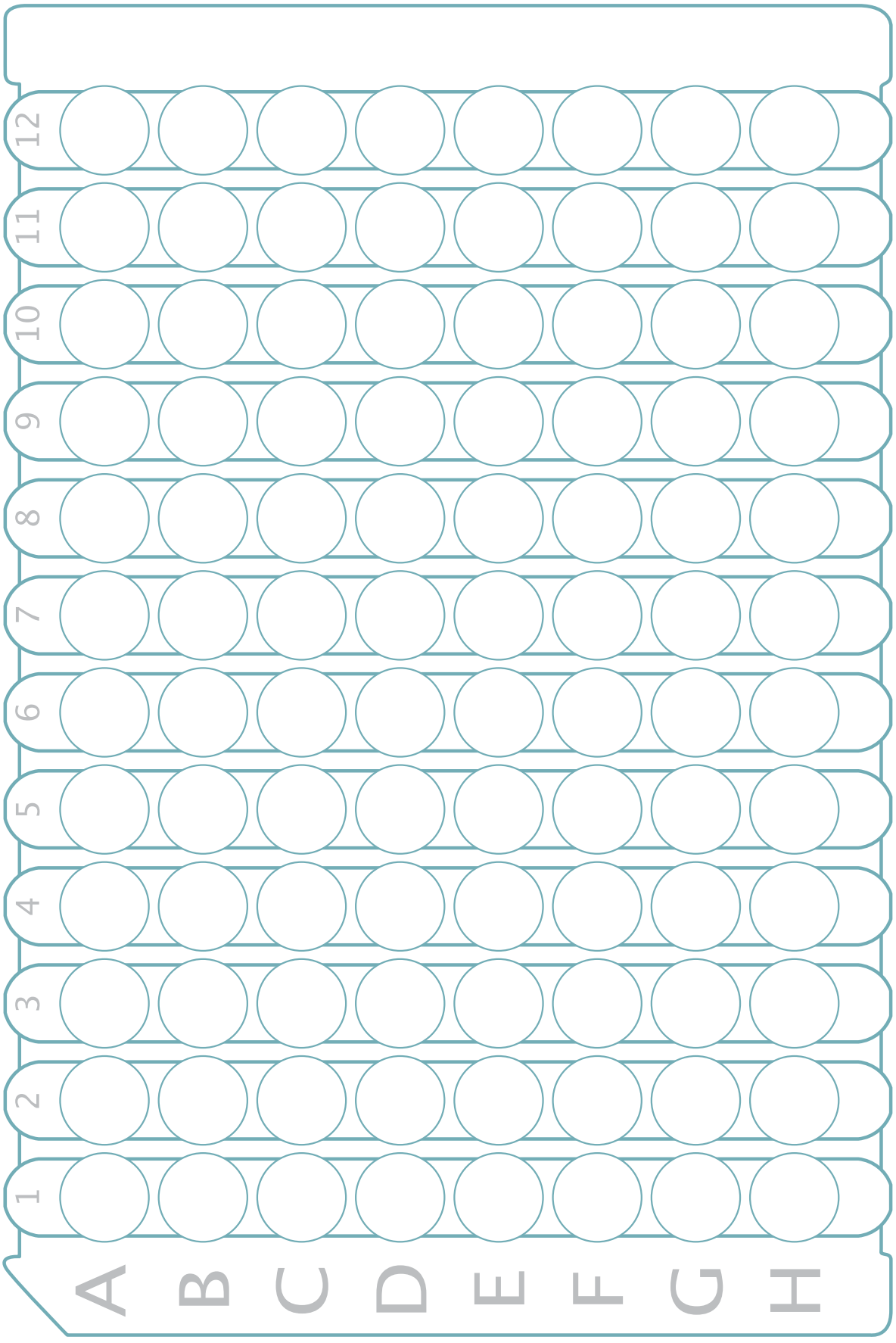
SPECIFICITY *CONTINUED*

Recombinant multiplex partners:

BDNF
CCL2/MCP-1
CCL4/MIP-1β
CCL5/RANTES
CCL11/Eotaxin
CCL20/MIP-3α
CD40 Ligand
CXCL2/GROβ
CXCL10/IP-10
CXCL11/I-TAC
CXCL13/BLC
FGF basic
G-CSF
GM-CSF
Granzyme B
IFN-α
IFN-β
IFN-γ
IL-1β
IL-10
IL-12 p70
IL-13
IL-15
IL-17A
IL-2
IL-21
IL-4
IL-5
IL-6
IL-7
IL-8/CXCL8
PDGF-AA
PDGF-BB
PD-L1
TGF-α
TNF-α
VEGF

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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