Acute Phase Response
Cancer
Cardiovascular Disease
Cytokines, Chemokines, and Growth Factors
Neurology
Toxicology
Infectious Disease
Immunoglobulin Isotyping
Signal Transduction

Bio-Plex Pro Human Cytokine 48-Plex Screening Panel

MAGNETIC SEPARATION ENABLED

Basic FGF, CTACK, eotaxin, G-CSF, GM-CSF, GRO-α, HGF, ICAM-1,* IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-2Rα, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-16, IL-17A, IL-18, IP-10, LIF, MCP-1 (MCAF), MCP-3, M-CSF, MIF, MIG, MIP-1α, MIP-1β, β-NGF, PDGF-BB, RANTES, SCF, SCGF-β, SDF-1α, TNF-α, TNF-β, TRAIL, VCAM-1,* VEGF-A

*** Recommended serum sample dilution: 100-fold. Cannot be muliplexed with analytes in the 48-plex assay.**

- All-in-one 48-plex panel
- Single-level quality control
- Magnetic workflow

High-Performance Multiplex Screening Immunoassays for Research and Drug Discovery

Cytokines and chemokines are extracellular mediators and regulators within a signaling network between cells and are key modulators of inflammation, participating in acute and chronic conditions via a complex network of interactions. This panel integrates a network of biologically relevant cytokines and chemokines in a single assay, enabling you to interrogate 48 biomarkers simultaneously. This tool provides a comprehensive, robust solution for screening 48 biologically relevant analytes in a single well, accelerating breakthrough research and biomarker discovery. All assays in this panel have gone through a vigorous validation process to ensure the data are accurate and reproducible.

Assay Features

- Comprehensive panel coverage
- Broad dynamic range
- Best-in-class performance
- Reduced turnaround time
- Reproducible results
- Streamlined data analysis
- Flexibility

Rigorous Assay Validation

All Bio-Plex Pro Assays undergo rigorous evaluation that includes the following parameters:

- Specificity (cross-reactivity)
- Accuracy (recovery) in key sample matrices
- Inter- and intra-assay precision
- Sensitivity (limit of detection [LOD])
- Assay working range (lower and upper limits of quantification [LLOQ/ULOQ])
- Linearity of dilution
- Parallelism and matrix effect
- Performance characteristics in real samples (Figures 1 and 2)

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Assay Performance Definitions

The following parameters are indicative of assay performance, as shown in Table 1.

- **Assay working range** — the range of concentrations within which the assay is precise and accurate. Boundaries of the assay working range are defined by the LLOQ and ULOQ
- **Precision** — the percentage coefficient of variation (%CV) at concentrations within the assay working range
- **Accuracy (recovery)** — percentage of the observed concentration relative to the expected concentration of a known amount of analyte within the assay working range
- **Sensitivity (LOD)** — the concentration of analyte for which the fluorescence intensity signal is 2 standard deviations above the background signal
- **Linearity of dilution** — determines to what extent the dosage response above LLOQ can be diluted and measured accurately within the assay working range (Table 2)
- **Parallelism** — determines if a diluent matrix used to construct a standard curve is biologically comparable to the diluent matrix used for sample preparation. This ensures that the recombinant calibrator shares the same binding characteristic to the antibodies as the native molecules (Table 3 and Figure 3)

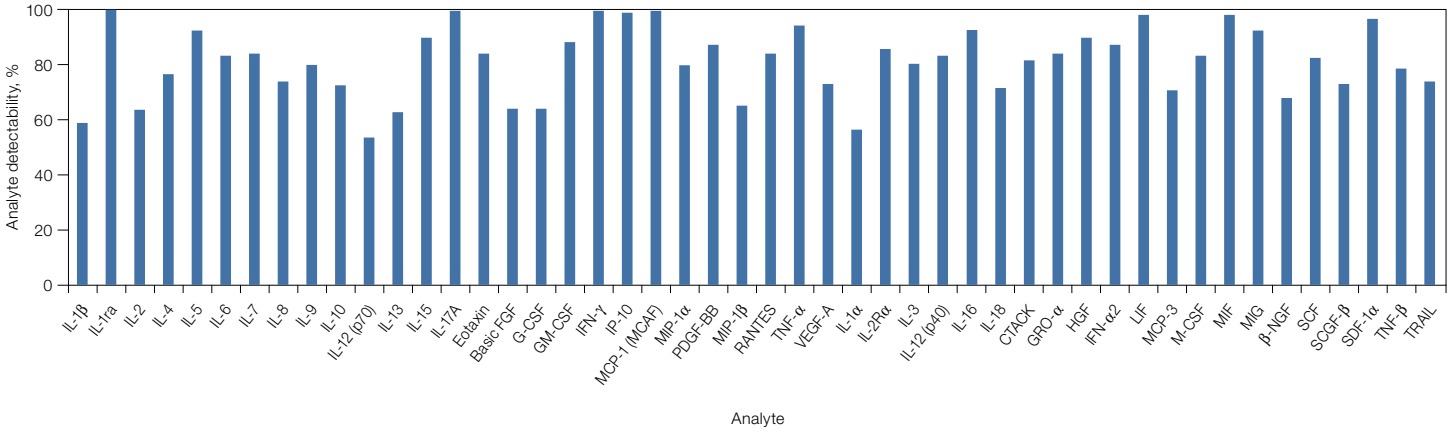

Fig. 1. Analyte detectability. Four hundred and twenty test samples from six laboratories, including culture media, cancer serum, stimulated peripheral blood mononuclear cells, whole blood, and human immunodeficiency virus plasma, were run on the assay. The average analyte detectability for the panel is >80%. Analyte detectability is defined as the percentage of analytes detected within the working assay range for all samples in a study.

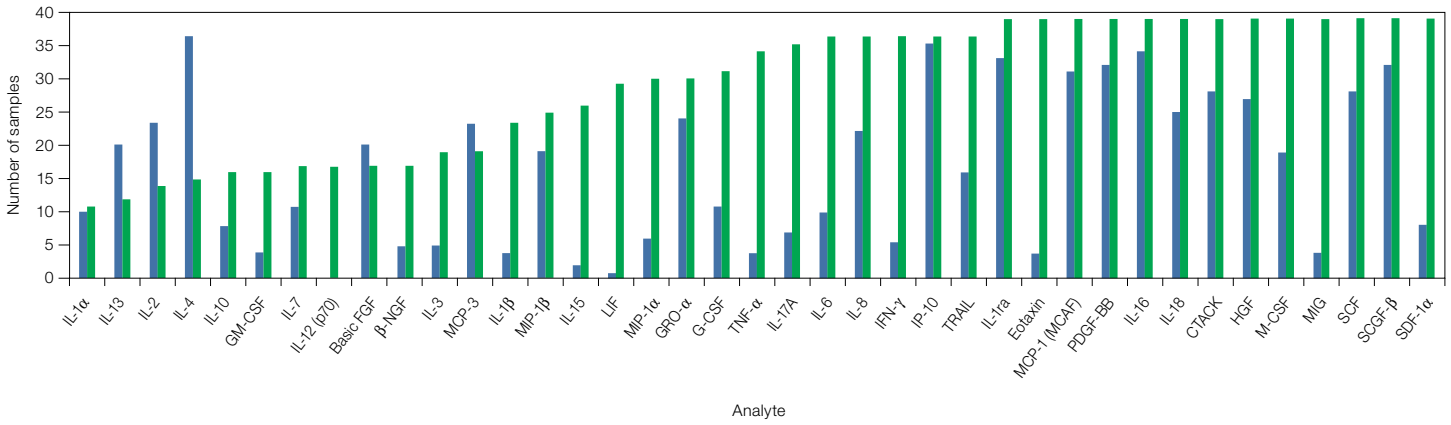

Fig. 2. Competitive comparison. Thirty-nine test samples were run on Vendor R's 39-plex and compared to the same overlapping 39 analytes in the Bio-Plex Pro Human Cytokine 48-Plex Screening Panel. This test configuration generated 1,521 total possible data points (39 samples x 39 analytes). In total, Vendor R's assay detected 637 data points within their working assay range while the 39 analytes from the Bio-Plex Pro Panel detected 1,140 data points. Detected data points divided by total possible data points defines analyte detectability in this comparison. Analyte detectability for Vendor R (■) is 42% while analyte detectability for the Bio-Plex Pro Panel (■) is 75%. Bio-Rad offers 79% improved analyte detectability over Vendor R in the context of these 39 overlapping targets.

Table 1. Representative performance characteristics.

Analyte	Assay Working Range, pg/ml		Assay Sensitivity, pg/ml	Mean Intra-Assay %CV*	Mean Inter-Assay %CV*	Singleplex Bead Region
	LLOQ*	ULOQ*	LOD*			
Basic FGF	3.26	3,341	2.54	3.1	2.4	44
CTACK	2.10	15,656	0.82	2.7	5.2	72
Eotaxin	0.14	2,281	0.05	4.4	1.2	43
G-CSF	6.35	104,106	3.63	3.1	4.0	57
GM-CSF	0.48	7,846	0.19	4.3	2.2	34
GRO-α	21.05	31,255	13.45	2.6	7.9	61
HGF	8.76	143,513	7.09	2.6	2.9	62
ICAM-1	3.84	62,935	1.51	4.4	2.3	12
IFN-α2	0.95	15,569	0.46	3.3	4.4	20
IFN-γ	1.57	25,665	1.05	3.1	3.6	21
IL-1α	3.73	61,154	6.65	3.5	4.9	63
IL-1β	0.29	4,672	0.24	3.6	3.2	39
IL-1ra	6.21	34,949	3.16	4.7	5.1	25
IL-2	1.29	21,178	0.75	1.7	2.5	38
IL-2Rα	1.48	24,270	1.65	3.4	4.8	13
IL-3	0.13	2,139	0.13	5.0	3.9	64
IL-4	0.19	3,064	0.09	3.2	1.9	52
IL-5	3.63	59,499	0.86	2.3	2.3	33
IL-6	0.38	6,244	0.34	2.2	3.0	19
IL-7	1.92	31,475	1.22	2.7	3.9	74
IL-8	0.85	13,992	0.36	3.2	2.8	54
IL-9	3.62	31,527	1.08	2.6	7.1	77
IL-10	1.06	17,427	0.69	2.3	3.4	56
IL-12 (p40)	14.68	240,582	6.39	4.5	2.4	28
IL-12 (p70)	1.43	23,425	0.78	3.3	2.9	75
IL-13	0.31	5,157	0.22	3.1	2.7	51
IL-15	12.42	203,426	12.82	2.8	4.1	73
IL-16	1.20	19,639	0.25	2.5	3.0	27
IL-17A	2.44	39,972	1.16	2.4	1.4	76
IL-18	0.66	10,892	0.31	2.9	2.2	42
IP-10	3.41	34,953	1.43	2.8	6.0	48
LIF	3.86	53,806	2.05	2.5	4.7	29
MCP-1 (MCAF)	0.53	8,755	0.44	3.2	3.4	53
MCP-3	0.48	4,899	0.24	4.4	4.2	26
M-CSF	0.75	12,290	0.27	2.4	3.6	67
MIF	2.70	44,168	2.45	3.4	4.7	35
MIG	3.16	32,365	1.39	4.4	4.2	14
MIP-1α	0.12	1,218	0.06	4.5	4.2	55
MIP-1β	1.41	1,439	1.41	3.4	2.5	18
β-NGF	0.47	7,655	0.23	2.9	3.9	46
PDGF-BB	7.12	37,133	2.96	3.3	9.7	47
RANTES	16.72	26,467	3.98	3.0	6.7	37
SCF	1.82	29,899	0.99	4.1	2.6	65
SCGF-β	82.11	1,345,200	141.77	2.3	3.8	78
SDF-1α	7.54	9,381	2.44	2.2	5.4	22
TNF-α	3.33	54,566	1.13	3.5	3.0	36
TNF-β	0.80	13,186	0.38	3.0	4.7	30
TRAIL	1.78	29,188	0.89	3.2	4.5	66
VCAM-1	2.91	47,652	2.05	4.1	3.1	15
VEGF-A	18.01	149,830	10.16	2.8	8.5	45

* The LLOQ, ULOQ, LOD, and inter-assay precision %CV are mean data determined from three independent multiplex assays in a serum-based matrix. Intra-assay %CV is derived from one representative assay. LLOQ and ULOQ are defined as the boundary standard curve points within which the performance specifications of individual standard points were met for a 10% intra-assay CV and recovery range of 70–130%. Data were generated using the magnetic workflow with the Bio-Plex Pro Wash Station.

Table 2. Linearity of dilution.*

Analyte	Linear Dilution Range	Analyte	Linear Dilution Range
Basic FGF	1:9–1:243	IL-13	1–1:2,187
CTACK	1–1:27**	IL-15	1:3–1:81
Eotaxin	1–1:243	IL-16	1–1:243
G-CSF	1–1:243	IL-17A	1–1:243
GM-CSF	1–1:2,187	IL-18	1–1:9
GRO-α	1:27–1:2,187	IP-10	1–1:81
HGF	1–1:81	LIF	1–1:729
IFN-α2	1:3–1:2,187	MCP-1 (MCAF)	1–1:81
IFN-γ	1–1:2,187	MCP-3	1–1:2,187
IL-1α	1–1:729	M-CSF	1–1:243
IL-1β	1–1:2,187	MIF	1:3–1:81
IL-1ra	1:3–1:243	MIG	1–1:81
IL-2	1–1:2,187	MIP-1α	1:3–1:243
IL-2Rα	1–1:81	MIP-1β	1:3–1:242
IL-3	1–1:729	β-NGF	1–1:729
IL-4	1–1:2,187	PDGF-BB	1:3–1:81
IL-5	1–1:729	RANTES	1–1:9
IL-6	1–1:81	SCF	1–1:243
IL-7	1:9–1:729	SCGF-β	***
IL-8	1–1:243	SDF-1α	***
IL-9	1:9–1:729	TNF-α	1–1:729
IL-10	1–1:729	TNF-β	1–1:2,187
IL-12 (p40)	1–1:2,187	TRAIL	1–1:243
IL-12 (p70)	1–1:2,187	VEGF-A	1:3–1:243

* Linearity of dilution determines whether the dosage response of the analyte is linear within the working assay range. A 50-plex recombinant standard was spiked into a serum sample which was previously diluted 1:4 with standard diluent HB then serially diluted seven times. Table 2 shows the sample dilution range where the dosage response is linear. The range is defined as the recovery of calculated concentrations within 70–130%.

** Recovery was 69% for CTACK at a 1:9 dilution.

*** Both SCGF-β and SDF-1α had high endogenous levels in the serum used in this study and were excluded.

Table 3. Parallelism.*

Analyte	%CV
CTACK	21
HGF	9
IL-16	7
IL-18	15
IP-10	12
MCP-1 (MCAF)	13
M-CSF	21
MIF	16
MIG	9
PDGF-BB	22
RANTES	19
SCF	19
SCGF-β	15
TRAIL	11

* Parallelism "is to ascertain that the binding characteristic of the endogenous analyte to the antibodies is the same as for the calibrator" (Andreasson et al. 2015). One human serum sample with high endogenous analytes was serially diluted (1:2). Table 3 shows the %CV of quantitation results from 25% serum samples and the subsequent dilutions.

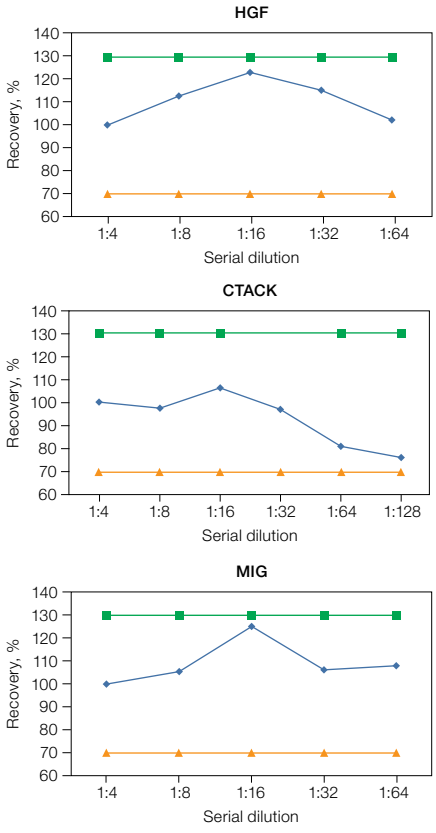

Fig. 3. Parallelism. Representative percentage recovery data from Table 3. Upper limit (■); human serum sample (♦); lower limit (▲).

Ordering Information

Catalog #	Description
12007283	**Bio-Plex Pro Human Cytokine 48-Plex Screening Panel**, 1 x 96-well, includes coupled magnetic capture beads, premixed detection antibodies, standards, quality control, detection antibody diluent HB, standard diluent HB, sample diluent HB, assay buffer, 10x wash buffer, streptavidin-PE, 96-well flat bottom plate, sealing tape, and instructions, for the detection of basic FGF, CTACK, eotaxin, G-CSF, GM-CSF, GRO-α, HGF, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-2Rα, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-16, IL-17A, IL-18, IP-10, LIF, MCP-1 (MCAF), MCP-3, M-CSF, MIF, MIG, MIP-1α, MIP-1β, β-NGF, PDGF-BB, RANTES, SCF, SCGF-β, SDF-1α, TNF-α, TNF-β, TRAIL, VEGF-A

Bio-Plex Pro Human Cytokine Singleplex Assays

171B5016M	**Basic FGF Set**	171B5012M	**IL-13 Set**
171B6006M	**CTACK Set**	171B5013M	**IL-15 Set**
171B5015M	**Eotaxin Set**	171B6005M	**IL-16 Set**
171B5017M	**G-CSF Set**	171B5014M	**IL-17A Set**
171B5018M	**GM-CSF Set**	171B5020M	**IP-10 Set**
171B6007M	**GRO-α Set**	171B6011M	**LIF Set**
171B6008M	**HGF Set**	171B5021M	**MCP-1 (MCAF) Set**
171B6009M	**ICAM-1 Set***	171B6012M	**MCP-3 Set**
171B6010M	**IFN-α2 Set**	171B6013M	**M-CSF Set**
171B5019M	**IFN-γ Set**	171B6014M	**MIF Set**
171B6001M	**IL-1α Set**	171B6015M	**MIG Set**
171B5001M	**IL-1β Set**	171B5022M	**MIP-1α Set**
171B5002M	**IL-1ra Set**	171B5023M	**MIP-1β Set**
171B5003M	**IL-2 Set**	171B6016M	**β-NGF Set**
171B6002M	**IL-2Rα Set**	171B5024M	**PDGF-BB Set**
171B6003M	**IL-3 Set**	171B5025M	**RANTES Set**
171B5004M	**IL-4 Set**	171B6017M	**SCF Set**
171B5005M	**IL-5 Set**	171B6018M	**SCGF-β Set**
171B5006M	**IL-6 Set**	171B6019M	**SDF-1α Set**
171B5007M	**IL-7 Set**	171B5026M	**TNF-α Set**
171B5008M	**IL-8 Set**	171B6020M	**TNF-β Set**
171B5009M	**IL-9 Set**	171B6021M	**TRAIL Set**
171B5010M	**IL-10 Set**	171B6022M	**VCAM-1 Set***
171B6004M	**IL-12 (p40) Set**	171B5027M	**VEGF-A Set**
171B5011M	**IL-12 (p70) Set**		

* Recommended serum sample dilution: 100-fold. Cannot be multiplexed with analytes in the 48-plex assay.

Reagent Kits

171304090M	**Bio-Plex Pro Reagent Kit III**, 1 x 96-well, includes detection antibody diluent HB, standard diluent HB, sample diluent HB, assay buffer, 10x wash buffer, streptavidin-PE, flat bottom plate, and sealing tape
12005846	**Bio-Plex Pro Reagent Kit III**, 10 x 96-well, includes detection antibody diluent HB, standard diluent HB, sample diluent HB, assay buffer, 10X wash buffer, streptavidin-PE, flat bottom plate, and sealing tape

Standards

12007919	**Bio-Plex Pro Human Cytokine Screening Panel Standards**, 1 vial, lyophilized mixture of 50 standard analytes, includes basic FGF, CTACK, eotaxin, G-CSF, GM-CSF, GRO-α, HGF, ICAM-1, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-2Rα, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-16, IL-17A, IL-18, IP-10, LIF, MCP-1 (MCAF), MCP-3, M-CSF, MIF, MIG, MIP-1α, MIP-1β, β-NGF, PDGF-BB, RANTES, SCF, SCGF-β, SDF-1α, TNF-α, TNF-β, TRAIL, VCAM-1, VEGF-A

Wash Stations and Accessories

30034376	**Bio-Plex Pro Wash Station**, microplate wash station for magnetic bead–based assays, includes magnetic plate carrier, waste bottle, 2 buffer bottles
171020100	**Bio-Plex Handheld Magnetic Washer**, includes magnetic washer and adjustment hex tools for use in manual wash steps for all Bio-Plex Magnetic Assays
171025001	**Bio-Plex Pro Flat Bottom Plates**, pkg of 40 x 96-well plates, for use with Bio-Plex Pro Wash Stations when using magnetic bead–based assays

Catalog #	Description

Software

171001510	**Bio-Plex Data Pro Software with Bio-Plex Manager Software**, Bio-Plex Data Pro Software (5 seats) for multi-experiment analysis and advanced data visualization and Bio-Plex Manager Software (5 seats) for instrument data evaluation and optimization; CDs and security HASP key included
171001513	**Bio-Plex Data Pro Software**, 5 seats, for multi-experiment analysis and advanced data visualization
171STND01	**Bio-Plex Manager Software**, 1 user desktop license, for analysis of Bio-Plex data and generation of protocols; does not operate the instrument

Additional Recommended Bio-Plex Assays for Studying Human Cytokines

171AL001M	**Bio-Plex Pro Human Inflammation 37-Plex Panel 1**, for the detection of APRIL/TNFSF13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, chitinase 3-like 1, gp130/sIL-6Rβ, IFN-α2, IFN-β, IFN-γ, IL-2, sIL-6Rα, IL-8, IL-10, IL-11, IL-12 (p40), IL-12 (p70), IL-19, IL-20, IL-22, IL-26, IL-27 (p28), IL-28A/IFN-λ2, IL-29/IFN-λ1, IL-32, IL-34, IL-35, LIGHT/TNFSF14, MMP-1, MMP-2, MMP-3, osteocalcin, osteopontin, pentraxin-3, sTNF-R1, sTNF-R2, TSLP, TWEAK/TNFSF12
171AK99MR2	**Bio-Plex Pro Human Chemokine 40-Plex Panel**, for the detection of 6Ckine/CCL21, BCA-1/CXCL13, CTACK/CCL27, ENA-78/CXCL5, eotaxin/CCL11, eotaxin-2/CCL24, eotaxin-3/CCL26, fractalkine/CX3CL1, GCP-2/CXCL6, GM-CSF, GRO-α/CXCL1, GRO-β/CXCL2, I-309/CCL1, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8/CXCL8, IL-10, IL-16, IP-10/CXCL10, I-TAC/ CXCL11, MCP-1/CCL2, MCP-2/CCL8, MCP-3/CCL7, MCP-4/CCL13, MDC/CCL22, MIF, MIG/CXCL9, MIP-1α/CCL3, MIP-1δ/CCL15, MIP-3α/CCL20, MIP-3β/CCL19, MPIF-1/CCL23, SCYB16/CXCL16, SDF-1α+β/CXCL12, TARC/CCL17, TECK/CCL25, TNF-α
171AA001M	**Bio-Plex Pro Human Th17 Cytokine 15-Plex Panel**, for the detection of IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-17A/F (singleplex only), IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN-γ, sCD40L,TNF-α
171W4001M	**Bio-Plex Pro TGF-β 3-Plex Panel**, for the detection of TGF-β1, TGF-β2, TGF-β3

Reference

Andreasson U et al. (2015). A practical guide to immunoassay method validation. Front Neurol 6, 179.

Visit **bio-rad.com/HCS** for more information.

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Life Science Group

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