

Bio-Plex Pro™ Human Cancer Biomarker Assays

Angiopoietin-2, sCD40L, EGF, sEGFR, Endoglin, sFASL, FGF-basic, Follistatin, G-CSF, HB-EG, sHER-2/neu, HGF, IGFBP-1, IL-6, sIL-6R α , IL-8, IL-18, Leptin, Osteopontin, PAI-1, PDGF-AB/BB, PECAM-1, PLGF, Prolactin, SCF, TGF- α , sTIE-2, TNF- α , uPA, VEGF-A, VEGF-C, VEGF-D, sVEGFR-1, sVEGFR-2

MAGNETIC SEPARATION ENABLED

- Validated on human plasma, serum, and cell culture samples
- Magnetic or vacuum assay separation
- All-in-one kit format
- Flexible ordering options



High-Performance Multiplex Immunoassays for Cancer Research

Bio-Plex Pro human cancer biomarker assays are a unique blend of magnetic bead-based assays designed to meet the needs of the most discerning preclinical and clinical researchers. The multiplex format enables robust and reproducible measurement of 34 biomarkers involved in:

- Angiogenesis
- Metastasis
- Cell proliferation
- Cell adhesion/migration
- Apoptosis
- Inflammation

The assays incorporate several new features to enhance both quality and ease of use:

- Reagent kit II for reliable performance in serum and plasma samples
- Optimized protocol for high precision and broad assay working ranges
- Assay quick guide to get you started right away
- 2-level quality controls included with premixed kits

Benefits of Magnetic Bead-Based Assays

Magnetic bead-based assays enable automation of wash steps with a Bio-Plex Pro series or similar wash station. This innovation greatly simplifies assay processing and eliminates the need for a vacuum manifold. After adopting the magnetic assay workflow, many users experience improved assay precision, in particular with viscous samples.

Rigorous Assay Validation

All Bio-Plex Pro assays are subjected to a rigorous evaluation that includes the following assay parameters:

- Specificity and cross-reactivity
- Accuracy (recovery) in key sample matrices
- Inter- and intra-assay precision
- Sensitivity (limit of detection, LOD)
- Assay working range (LLOQ/ULOQ)
- Linearity of dilution
- Parallelism and matrix effect
- Sample analysis ensuring normal and disease samples fall within the assay range

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 lower limit of quantitation (LLOQ) and the upper limit of quantitation (ULOQ)

Precision – the 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 (limit of detection, LOD) – the concentration of analyte for which the fluorescence intensity signal is two standard deviations above the background signal

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Table 1. Representative assay performance.

Target	Alternate Names	Assay Working Range, pg/ml		Assay Sensitivity, pg/ml	Assay Precision		Calibration to WHO/NIBSC		
		LLOQ	ULOQ	LOD	Intra-assay %CV	Inter-assay %CV	Factor*	NIBSC Catalog #	
Human Cancer Biomarker Panel 1 — Receptors, cytokines, chemokines, growth factors, and hormones									
sEGFR	ErbB1, EGFR1	11.9	195,000	3.5	3.6	3.8	—	—	
FGF-basic	FGF2, BFGF	10.2	4,500	5.1	2.9	4.7	6.6	90/712	
Follistatin	FSN	4.6	42,750	1.5	3.1	3.6	—	—	
G-CSF	—	1.1	18,000	1.1	3.7	7.7	0.8	09/136	
HGF	SF	4.5	42,124	1.8	3.2	6.1	0.7	96/564	
sHER-2/neu	ErbB2	1.4	23,500	0.5	3.2	3.9	—	—	
sIL-6R α	CD126	1.4	12,985	0.5	3.1	5.3	—	—	
Leptin	—	7.3	119,429	1.1	2.8	9.6	1.2	97/594	
Osteopontin	OPN	72.5	216,000	56.4	4.0	7.8	—	—	
PECAM-1	CD31	51.5	135,000	24.4	4.2	5.9	—	—	
PDGF-AB/BB	—	2.1	35,000	0.7	3.2	7.9	1.8	94/728	
Prolactin	PRL	12.2	200,000	7.0	3.0	6.8	1.5	98/582	
SCF	KL1	2.0	32,000	0.1	2.9	3.6	1.3	91/682	
sTIE-2	CD202b	12.2	200,000	6.6	3.0	5.8	—	—	
sVEGFR-1	FLT1	4.3	35,324	0.8	2.9	3.3	—	—	
sVEGFR-2	KDR, FLK1	12.1	197,681	7.4	3.1	3.5	—	—	
Human Cancer Biomarker Panel 2 — Ligands for receptors in panel 1, cytokines, chemokines, and growth factors									
Angiotensin-2	—	19.5	51,100	3.3	2.4	6.1	—	—	
sCD40L	TRAP	1.4	18,405	0.3	2.0	4.1	—	—	
EGF	—	0.3	4,942	0.1	4.1	7.2	0.6	91/530	
Endoglin	CD105	3.3	54,740	1.0	2.9	9.6	—	—	
sFASL	CD95L/APO-1L	7.5	13,455	2.7	4.4	8.2	—	—	
HB-EGF	—	0.3	4,314	0.1	3.8	9.0	—	—	
IGFBP-1	—	4.2	39,514	1.5	3.2	4.7	—	—	
IL-6	—	0.7	11,489	0.1	2.7	6.4	1.7	89/548	
IL-8	CXCL8	0.4	4,943	0.1	3.0	4.9	1.0	89/520	
IL-18	—	1.6	25,798	0.3	1.8	7.2	1.8	03/200	
PAI-1	SERPINE1	3.7	59,826	0.2	2.5	5.9	—	—	
PLGF	PGF	0.6	9,590	0.2	2.5	6.8	0.4	09/272	
TGF- α	—	0.6	7,858	0.2	2.7	8.2	—	—	
TNF- α	—	0.6	9,572	0.2	3.4	6.3	0.4	88/786	
uPA	Urokinase	0.9	14,934	0.1	3.5	4.6	—	—	
VEGF-A	VPF	1.7	27,518	0.4	2.4	8.6	1.1	02/286	
VEGF-C	Flt4 ligand	3.6	58,521	2.3	3.4	3.0	—	—	
VEGF-D	FIGF	14.0	17,614	11.5	3.5	8.2	—	—	

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 in which the performance specifications of individual standard points were met for a 10% intra-assay CV and recovery range of 80–120%. Data were generated using the magnetic workflow with the Bio-Plex Pro II wash station.

* Factor \times NIBSC value (pg/ml) = Bio-Plex value (pg/ml). Factors are based on average % recovery of Bio-Plex standards (in the linear range) relative to NIBSC standards run on the same assay plate.

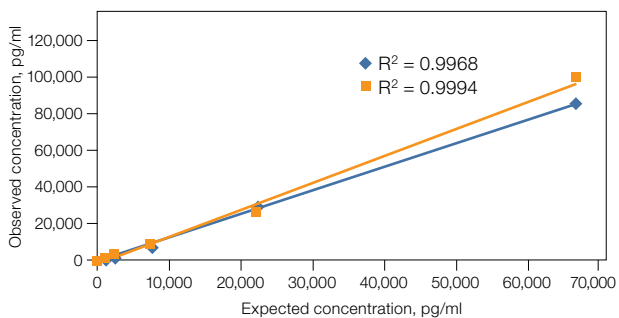


Fig. 1. Linearity of dilution determines the suitability of a standard curve for reflecting relative quantities of analyte in a complex matrix. Linearity of dilution of prolactin was assessed by spiking a known quantity of recombinant antigen into human serum and plasma matrices. ■, human serum; ◆, human plasma.

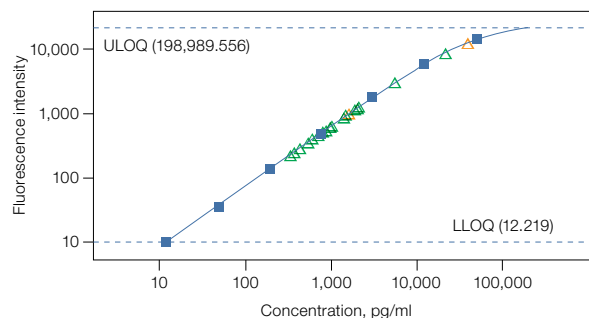


Fig. 2. Prolactin standard curve with human serum samples. Data were analyzed using Bio-Plex Manager™ software version 6.0. Recovery range specification was set to 80–120%. ■, standard; △, test samples; △, control.

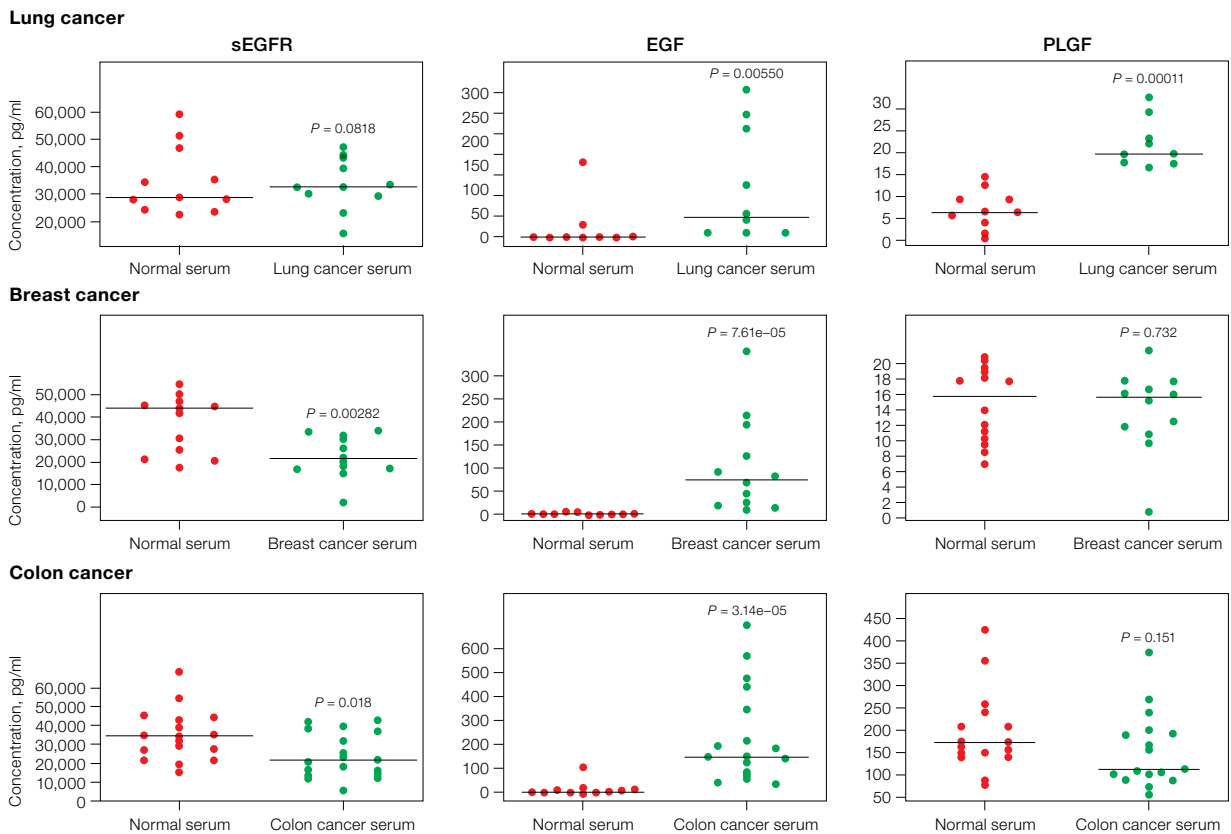


Fig. 3. Levels of biomarkers in normal (●) and cancer (●) groups. A Student's *t*-test was used to determine statistical significance between groups. Black lines denote mean values; *P* values are indicated above each cancer population. Data analysis, graphing, and statistics were performed with Bio-Plex Data Pro™ software.

Flexible Ordering Options

Premixed 16-Plex and 18-Plex Panels

This option provides excellent value and performance as well as fast delivery. The full panel is available as an all-in-one assay kit, which includes a flat bottom plate so that you can take advantage of magnetic assay processing.

x-Plex™ Custom Assay Service (We Mix)

A great choice for premium custom-mixed assays. Simply select your analytes of interest and plate type using the online Bio-Plex® assay builder at www.bio-rad.com/bio-plex/assaybuilder. Assays are mixed for you at Bio-Rad and delivered as an all-in-one kit.

Express Custom Assay Service (You Mix)

A fast and economical choice. Simply select your singleplex analytes of interest and plate type using the online Bio-Plex assay builder at www.bio-rad.com/assaybuilder. Assays are delivered as individual sets of coupled beads and detection antibodies in an all-in-one kit, ready for you to mix.

Individual Components

For your convenience, a host of singleplex sets and individual assay components are also available. See ordering information provided in this bulletin, or log on to www.bio-rad.com/bio-plex. Note: the quality controls provided in premixed assay kits are not sold separately.

For more information see Table 2.

Table 2. Comparison of features for each ordering option.

	Premixed 16-Plex and 18-Plex Panels	x-Plex Custom Assay (We Mix)	Express Custom Assay (You Mix)	Singleplex/Individual Components
All-in-one kit	•	•	•	
2-level quality controls	•	•		
Flat bottom plate only	•			
Choice of plate type		•	•	•
Choice of analytes		•	•	•
Detailed product data sheet	•	•		
Assay quick guide*	•	•	•	
Faster delivery	•		•	•
Premium performance	•	•		

* Assay quick guides and manuals are available online at www.bio-rad.com/bio-plex.

Ordering Information

Catalog# Description

Premixed, All-In-One Kit

Premixed panels include premixed coupled magnetic beads, detection antibodies, standards, 2-level controls, detection antibody diluent, standard diluent HB, sample diluent HB, assay buffer, wash buffer, streptavidin-PE, 96-well flat bottom plate, sealing tape, and instructions.

171-AC500M	Bio-Plex Pro Human Cancer Biomarker Panel 1, 16-Plex , 1 × 96-well, for the detection of sEGFR, FGF-basic, follistatin, G-CSF, sHER-2/neu, HGF, sIL-6R α , leptin, osteopontin, PDGF-AB/BB, PECAM-1, prolactin, SCF, sTIE-2, sVEGFR-1, sVEGFR-2
171-AC600M	Bio-Plex Pro Human Cancer Biomarker Panel 2, 18-Plex , 1 × 96-well, for the detection of angiotensin-2, sCD40L, EGF, endoglin, sFASL, HB-EGF, IGFBP-1, IL-6, IL-8, IL-18, PAI-1, PLGF, TGF- α , TNF- α , uPA, VEGF-A, VEGF-C, VEGF-D

Bio-Plex Pro Human Cancer Biomarker Panel 1 Singleplex Sets*

1 × 96-well, include coupled magnetic beads and detection antibodies. Require Bio-Plex Pro reagent kit II and panel 1 standards (171-DC5000).

171-BC501M	sEGFR Set
171-BC502M	FGF-basic Set
171-BC503M	Follistatin Set
171-BC504M	G-CSF Set
171-BC505M	HGF Set
171-BC506M	sHER-2/neu Set
171-BC507M	sIL-6Rα Set
171-BC508M	Leptin Set
171-BC509M	Osteopontin Set
171-BC510M	PECAM-1 Set
171-BC511M	PDGF-AB/BB Set
171-BC512M	Prolactin Set
171-BC513M	SCF Set
171-BC514M	sTIE-2 Set
171-BC515M	sVEGFR-1 Set
171-BC516M	sVEGFR-2 Set

Bio-Plex Pro Human Cancer Biomarker Panel 2 Singleplex Sets*

1 × 96-well, include coupled magnetic beads and detection antibodies. Require Bio-Plex Pro reagent kit II and panel 2 standards (171-DC6000).

171-BC601M	Angiotensin-2 Set
171-BC602M	sCD40L Set
171-BC603M	EGF Set
171-BC604M	Endoglin Set
171-BC605M	sFASL Set
171-BC606M	HB-EGF Set
171-BC607M	IGFBP-1 Set
171-BC608M	IL-6 Set
171-BC609M	IL-8 Set
171-BC611M	PAI-1 Set
171-BC612M	PLGF Set
171-BC613M	TGF-α Set
171-BC614M	TNF-α Set
171-BC615M	uPA Set
171-BC616M	VEGF-A Set
171-BC617M	VEGF-C Set
171-BC618M	VEGF-D Set

Catalog# Description

Reagent Kits

171-304055	Bio-Plex Pro Reagent Kit II with Filter Plate , 1 × 96-well, includes detection antibody diluent, standard diluent HB, sample diluent HB, assay and wash buffers, streptavidin-PE, filter plate, and sealing tape, for vacuum separation methods
171-304055M	Bio-Plex Pro Reagent Kit II with Flat Bottom Plate , 1 × 96-well, includes detection antibody diluent, standard diluent HB, sample diluent HB, assay and wash buffers, streptavidin-PE, flat bottom plate, and sealing tape, for magnetic separation methods

Standards

171-DC5000	Bio-Plex Pro Human Cancer Biomarker Panel 1, 16-Plex Standards , pkg of 1 vial, lyophilized mixture of 16 standard analytes
171-DC5001	Bio-Plex Pro Human Cancer Biomarker Panel 1, 16-Plex Standards , pkg of 50 lot-matched vials, lyophilized mixture of 16 standard analytes
171-DC6000	Bio-Plex Pro Human Cancer Biomarker Panel 2, 18-plex Standards , pkg of 1 vial, lyophilized mixture of 18 standard analytes
171-DC6001	Bio-Plex Pro Human Cancer Biomarker Panel 2, 18-plex Standards , pkg of 50 lot-matched vials, lyophilized mixture of 18 standard analytes

Wash Stations and Accessories

300-34376	Bio-Plex Pro Wash Station , includes magnetic plate carrier, waste bottle, 2 buffer bottles
300-34377	Bio-Plex Pro II Wash Station , includes magnetic plate carrier, vacuum manifold plate carrier, waste bottle, 2 buffer bottles
171-025001	Bio-Plex Pro Flat Bottom Plates , 40 × 96-well plates
171-304500	Bio-Plex Wash Buffer , 1.5 L
171-304502	Filter plate , pkg of 1, 96-well plate with clear plastic lid and tray, for Bio-Plex assays using the vacuum wash method, sealing tape not included
171-020100	Bio-Plex Handheld Magnetic Washer , includes magnetic washer and adjustment hex tools for use in manual wash steps for all Bio-Plex magnetic assays

Software

171-001513	Bio-Plex Data Pro Software (5 seats) , for multi-experiment analysis and advanced data visualization
171-001523	Bio-Plex Data Pro Plus Software , contains all the features of Bio-Plex Data Pro software with added visualization, sharing, and analysis functionality.
171-STND01	Bio-Plex Manager Software (1 seat) , for instrument data evaluation and optimization

The Bio-Plex multiplex system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.

* It is not recommended to mix assays between panel 1 and panel 2.



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