

Acute Phase Response  
 Cancer  
 Cardiovascular Disease  
 Diabetes  
 Cytokines, Chemokines, and Growth Factors  
 Immunology/Inflammation  
 Immunoglobulin Isotyping  
 Cell Signaling  
 Toxicology

# Bio-Plex Pro™ Human Inflammation Assays

APRIL / TNFSF13, BAFF / TNFSF13B, sCD30 / TNFRSF8, sCD163, Chitinase 3-like 1, gp130 / sIL-6R $\beta$ , IFN- $\alpha$ 2, IFN- $\beta$ , IFN- $\gamma$ , IL-2, sIL-6R $\alpha$ , 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- $\lambda$ 2, IL-29 / IFN- $\lambda$ 1, 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

MAGNETIC SEPARATION ENABLED

- Validated on plasma, serum, and cell culture samples
- Three distinct all-in-one multiplex kits
- Custom configurations
- Magnetic workflow



## High Performance Multiplex Assays for Immunology Research

The Bio-Plex Pro Human Inflammation Assays comprise the most relevant set of 37 inflammation biomarkers in a single multiplex assay for the study of inflammation-associated diseases. Inflammation is the hallmark of allergies, infectious diseases, and wound healing. It is a leading cause of mortality worldwide and is indicated in eight major disease areas, including:

- Autoimmune diseases
- Cancer
- Cardiovascular diseases
- Infectious diseases
- Diabetic complications
- Metabolic disorder complications
- Neurological diseases
- Pulmonary diseases

## Assay Features

- Unique and relevant mixture of targets, including key biomarkers from the TNF superfamily, Treg cytokines, IFN proteins, and matrix metalloproteinases (MMPs), in one panel
- Single-level quality control with kit lot-specific ranges
- Magnetic beads for simplified plate processing
- Single dilution factor for all targets
- Assay quick guide to get you started right away
- Compatible with Bio-Plex® 200, Bio-Plex® 3D, and Bio-Plex® MAGPIX™ Systems

## 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 (LLOQ/ULOQ)
- Linearity of dilution
- Parallelism and matrix effect
- Performance characteristics in real samples

## 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 quantification (LLOQ) and the upper limit of quantification (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 (LOD)** — the concentration of analyte for which the fluorescence intensity signal is two standard deviations above the background signal

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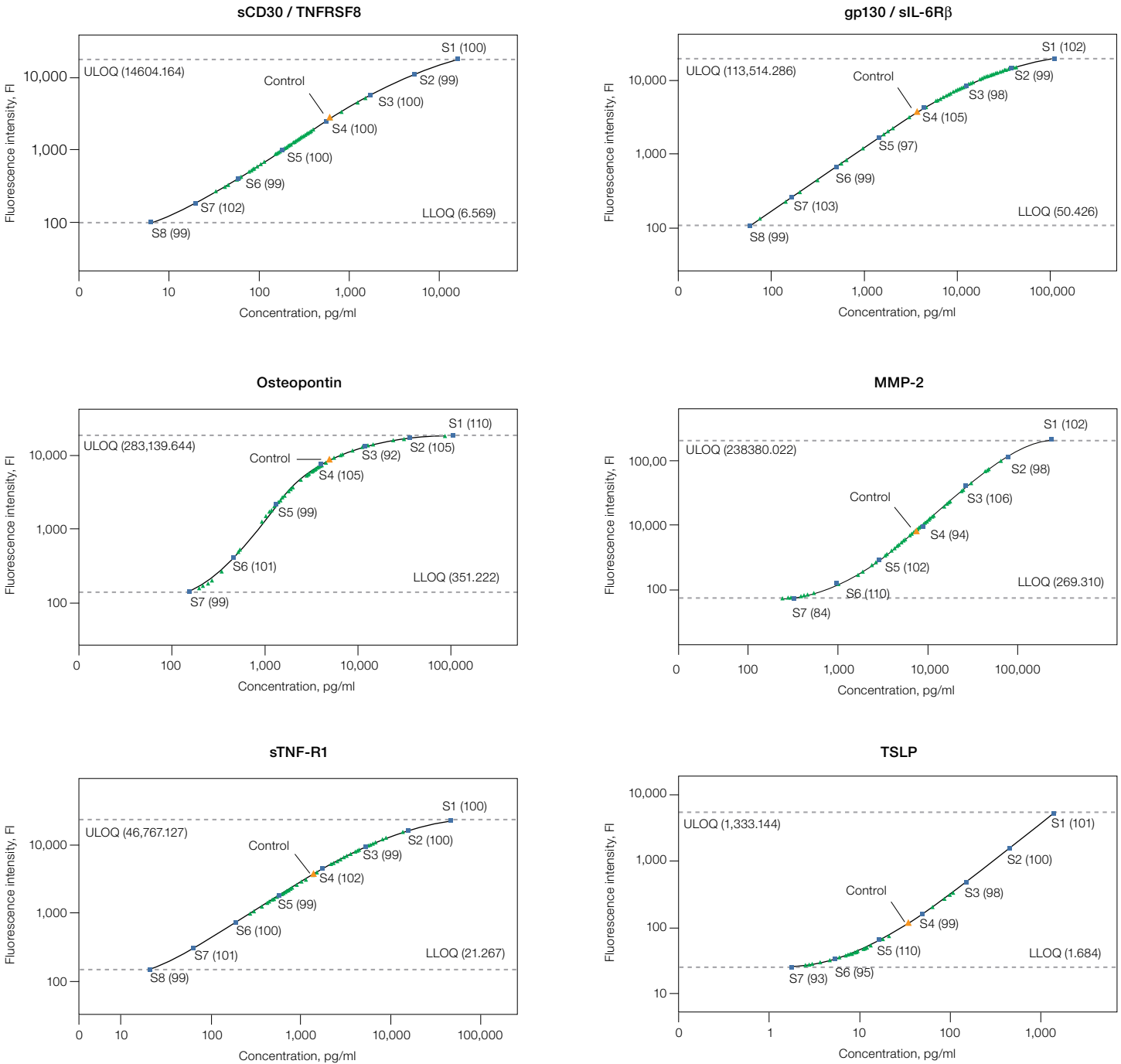
**Table 1. Representative performance characteristics.**

Analyte	Assay Working Range, pg/ml*		Assay Sensitivity, pg/ml*	Assay Precision*		37-Plex Panel 171-AL001M	24-Plex Panel 171-AL002M	Treg 12-Plex Panel 171-AL003M	Singleplex Bead Region
	LLOQ	ULOQ	LOD	Intra-Assay %CV	Inter-Assay %CV				
APRIL / TNFSF13	3,440.1	2,507,800.0	190.0	4.2	17.3	•	•		42
BAFF / TNFSF13B	91.5	200,008.9	34.7	1.6	5.2	•	•		37
sCD30 / TNFRSF8	53.4	12,973.1	1.0	2.5	5.0	•	•		53
sCD163	1,338.7	975,916.6	16.8	4.2	8.1	•	•		46
Chitinase 3-like 1	36.7	80,275.3	10.3	2.5	6.0	•	•		72
gp130 / sIL-6Rβ	257.6	187,785.6	16.9	2.3	5.9	•	•		14
IFN-α2	3.1	6,702.2	0.7	3.6	10.0	•			20
IFN-β	0.9	1,872.8	2.0	3.0	11.2	•	•		44
IFN-γ	6.3	13,694.9	0.05	3.7	7.3	•			21
IL-2	1.2	2,662.7	0.1	3.3	10.3	•		•	38
sIL-6Rα	18.6	40,675.1	1.5	3.1	3.2	•			19
IL-8	4.5	9,762.7	2.7	4.5	7.7	•			54
IL-10	1.7	3,781.2	0.6	3.0	8.5	•		•	56
IL-11	0.1	207.1	0.05	3.6	16.9	•	•		39
IL-12 (p40)	5.5	12,039.9	5.6	3.5	6.3	•		•	28
IL-12 (p70)	1.3	908.0	0.1	3.5	5.5	•		•	75
IL-19	8.5	6,223.8	0.2	3.8	8.4	•	•	•	29
IL-20	2.7	5,809.9	3.6	2.3	11.1	•	•	•	30
IL-22	5.3	11,511.0	1.1	3.2	6.5	•		•	18
IL-26	8.2	5,969.7	1.2	3.1	10.9	•	•	•	22
IL-27 (p28)	3.8	8,397.0	0.1	3.0	6.5	•	•	•	13
IL-28A / IFN-λ2	10.7	7,813.5	1.8	4.3	7.9	•	•	•	66
IL-29 / IFN-λ1	5.2	11,431.4	1.6	4.4	3.9	•	•	•	33
IL-32	3.9	8,586.5	12.3	2.5	8.8	•	•		35
IL-34	61.9	45,142.7	51.9	3.2	6.7	•	•		15
IL-35	20.6	45,037.6	3.7	2.6	9.1	•	•	•	34
LIGHT / TNFSF14	14.8	3,585.3	10.2	3.1	10.4	•	•		51
MMP-1	106.8	233,460.8	33.7	4.8	9.0	•			43
MMP-2	1,224.5	297,540.2	39.0	4.8	7.7	•			26
MMP-3	395.7	288,429.4	28.5	3.7	8.8	•			45
Osteocalcin	285.6	208,184.6	23.4	1.6	5.8	•	•		65
Osteopontin	115.3	252,154.4	91.3	2.3	4.5	•			77
Pentraxin-3	6.0	13,186.4	0.8	2.7	6.8	•	•		48
sTNF-R1	26.8	58,575.9	0.2	2.2	6.1	•	•		73
sTNF-R2	30.3	66,190.7	3.2	3.0	7.7	•	•		67
TSLP	3.0	2,165.0	0.8	3.3	9.4	•	•		52
TWEAK / TNFSF12	3.1	6,772.8	0.5	2.2	15.2	•	•		62

\* 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 70–130%. Data were generated using the magnetic workflow with the Bio-Plex Pro Wash Station.

## Bio-Plex Pro Assay Working Range

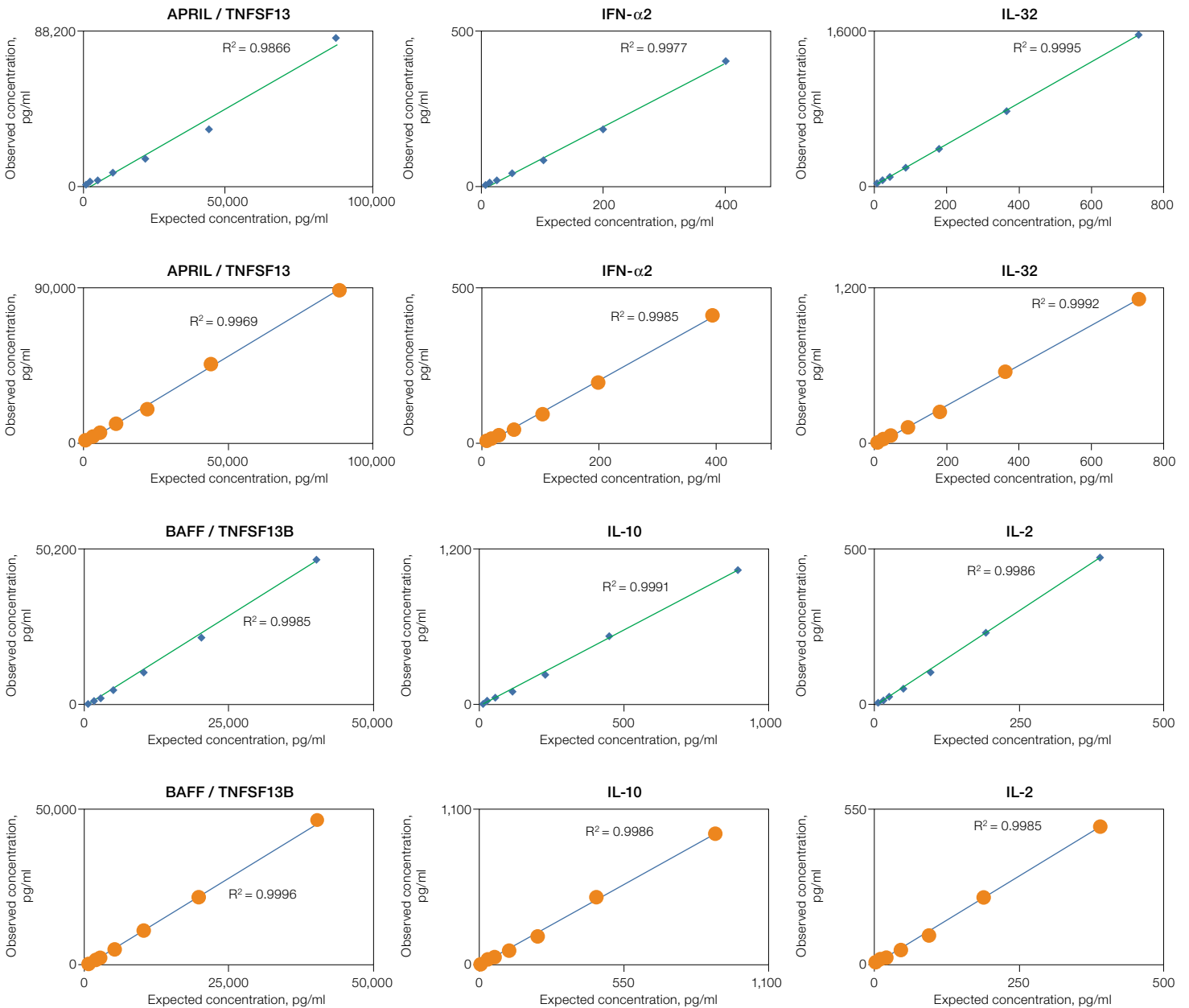
The assay working range should encompass the biological range of expression in order to be useful in research. Bio-Plex Pro Assays are developed and optimized to ensure real sample data fall within the quantifiable regions of the assay as demonstrated by comparing the standard curves of assay controls to biological samples (Figure 1).



**Fig. 1. Standard curves with assay controls and serum samples.** Standard points were prepared by serially diluting a reconstituted standard fourfold to generate an eight-point standard curve. Standard points with % recovery (■); samples (▲). Data were generated in Bio-Plex Manager™ Software.

## Accuracy of Bio-Plex Pro Human Inflammation Assays

Linearity of dilution displays the ability of an assay to generate measured values from complex samples by comparing the accuracy over a range of sample dilutions. Bio-Plex Pro Assays are designed and validated with high linearity to return accurate results from complex matrices (Figure 2).

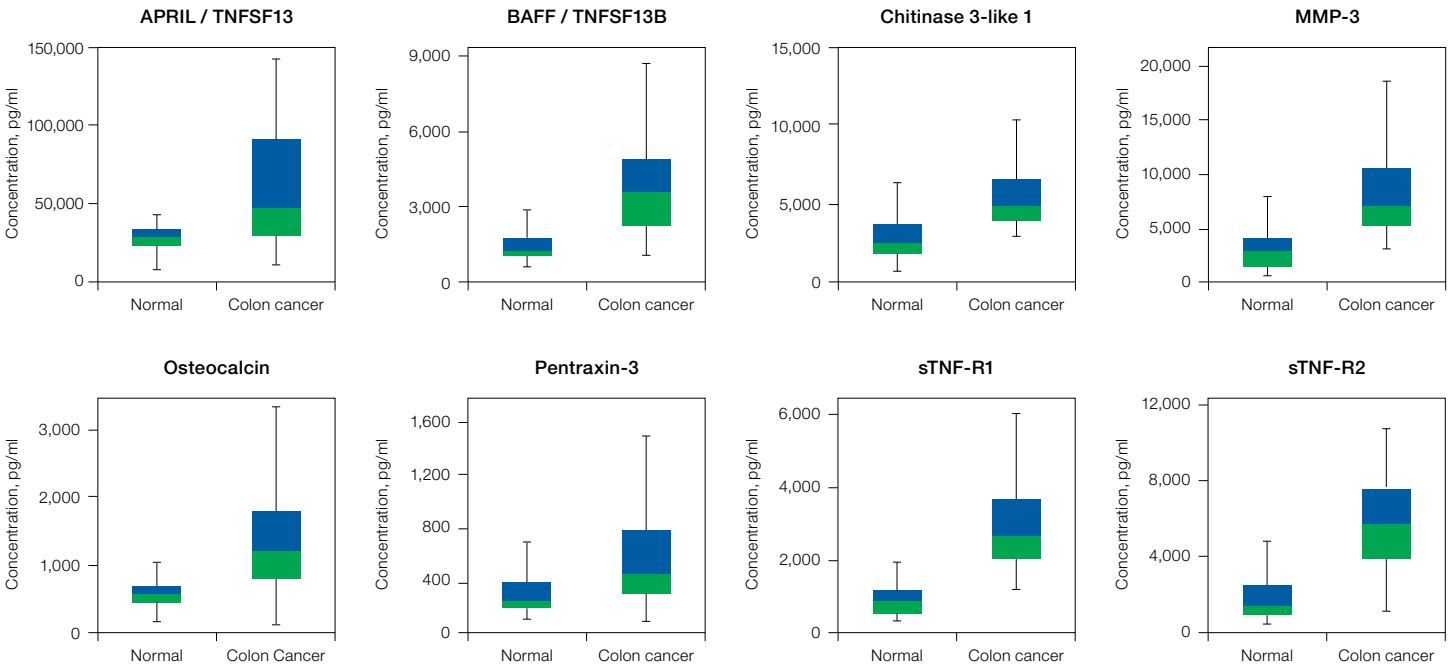


**Fig. 2. Linearity of dilution determines the suitability of a standard curve for reflecting relative quantities of an analyte in a complex matrix.** Linearity of dilution was assessed by spiking a known quantity of recombinant antigen into human serum and plasma matrices. Observed and expected analyte concentrations were plotted and the correlation coefficient ( $R^2$ ) values reflect linearity in signal response. Serum (◆); plasma (●).

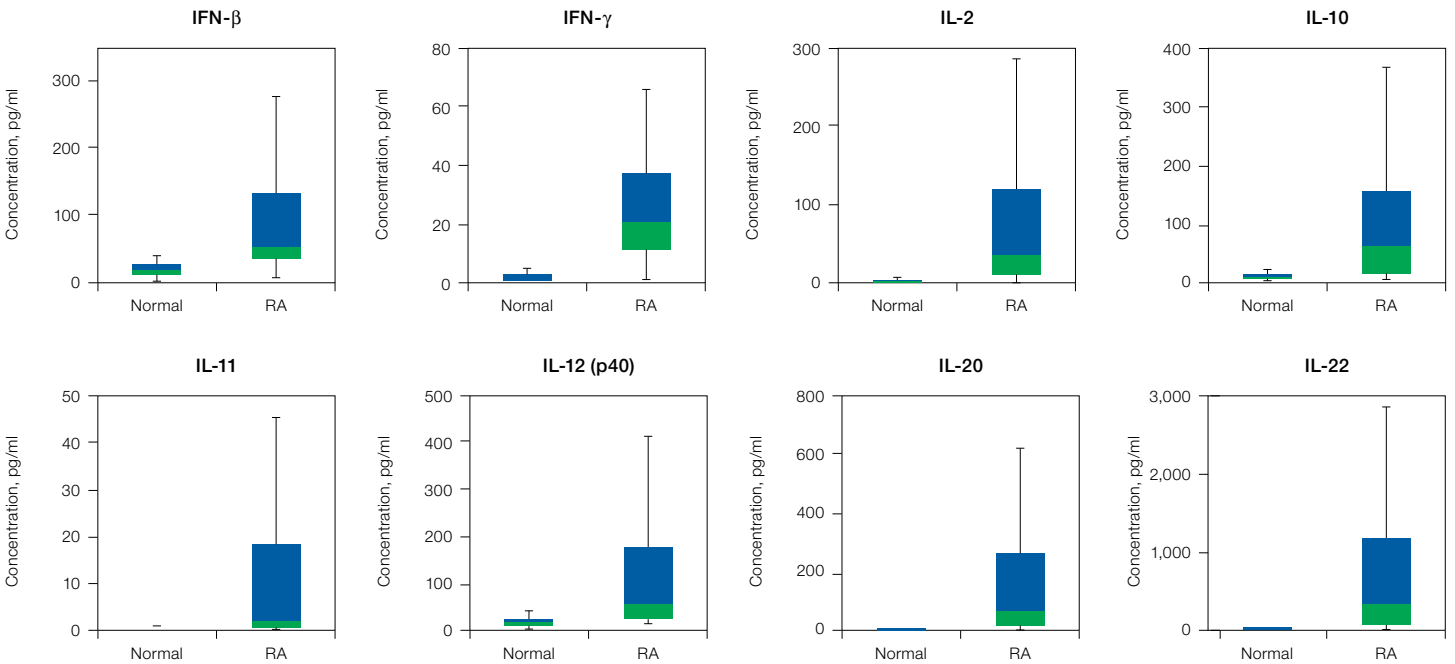
## Detection of Analytes

Bio-Plex Pro Assays are tested with samples from multiple sources to ensure target analytes are detected within normal biological expression levels and levels associated with disease (Figure 3).

### Colon Cancer

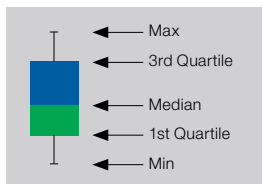
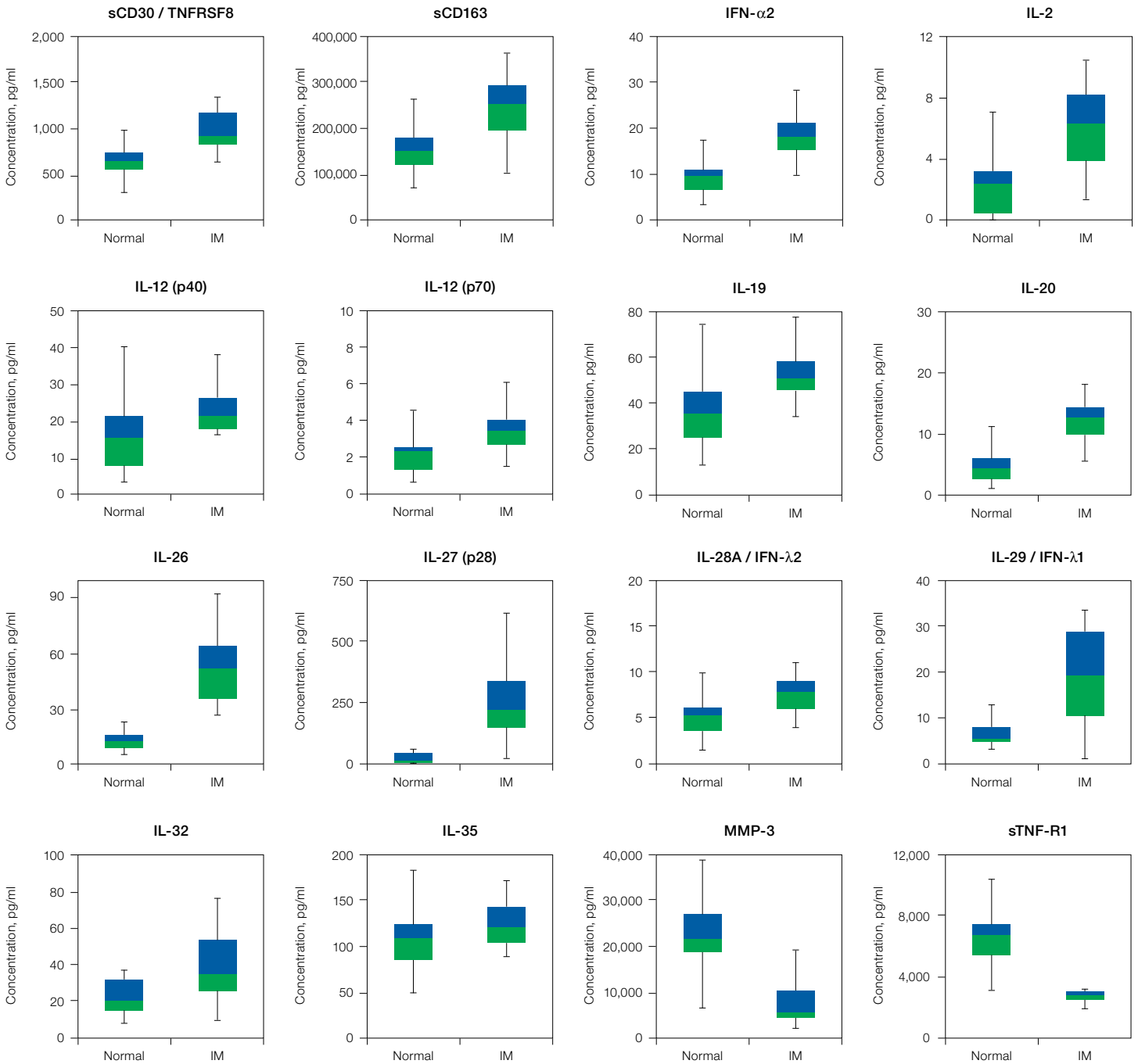


### Rheumatoid Arthritis (RA)



continues

**Infectious Mononucleosis (IM)**

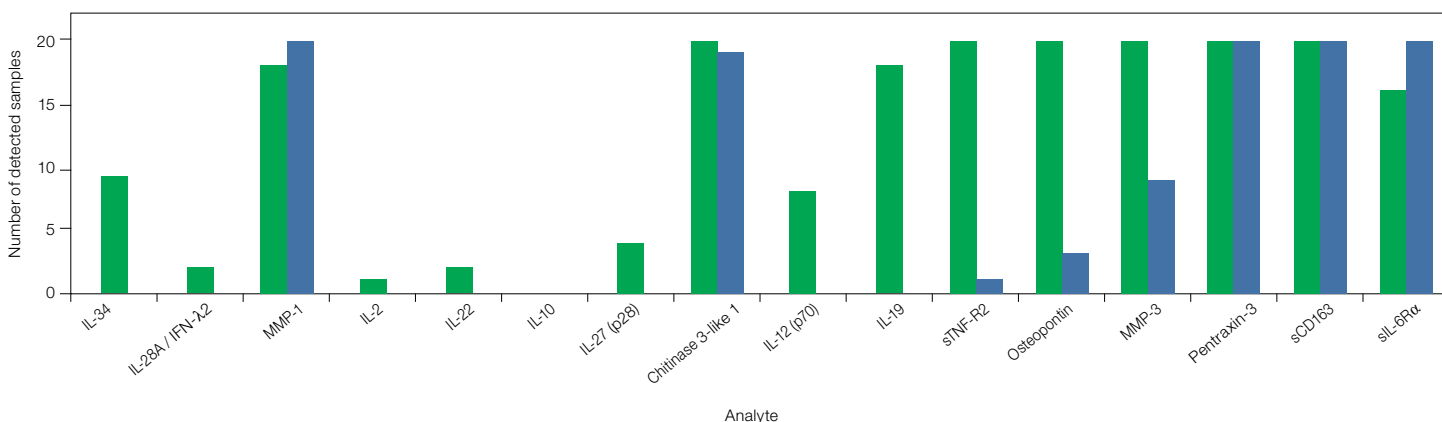


**Fig. 3. Box-and-whisker plots showing serum levels of biomarkers in normal and disease groups.** Colon cancer: normal serum n = 26, disease serum n = 17; rheumatoid arthritis (RA): normal serum n = 19, disease serum n = 120; infectious mononucleosis (IM): normal serum n = 19, disease serum n = 16. Please refer to the key on the left to interpret the plots.

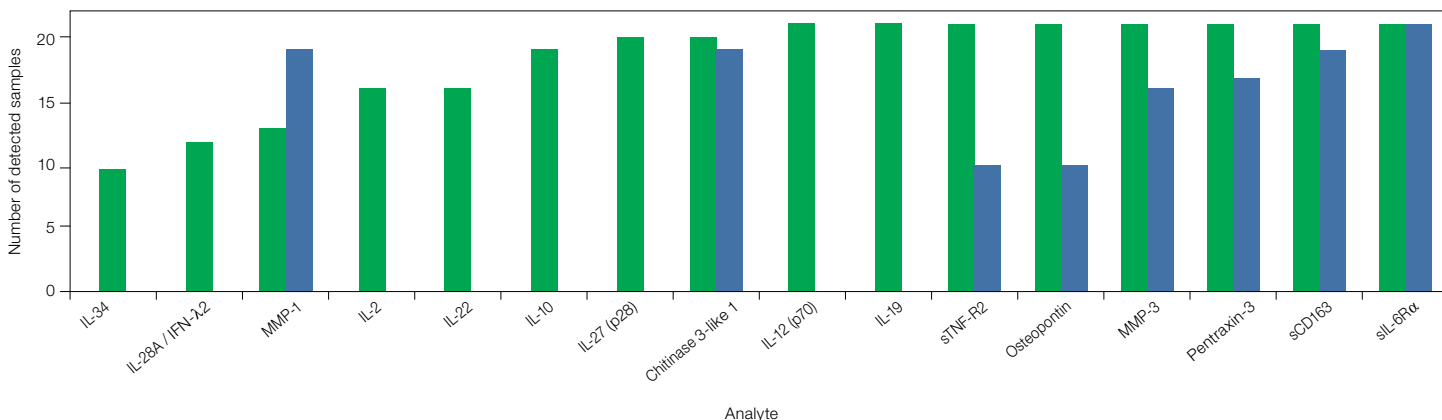
## Bio-Plex Pro Assays Provide More Reliable Sample Detection

The Bio-Plex Pro Human Inflammation Assays were compared to overlapping assays from vendor R to highlight more reliable sample detection in serum (Figure 4).

### Normal samples



### RA samples



**Fig. 4. Comparison of the Bio-Plex Pro Human Inflammation Assays to an equivalent multiplex assay from vendor R.** Serum samples from 20 normal donors (top panel) and 21 donors with severe RA (bottom panel) were collected and evaluated for the presence of various analytes. The number of samples that the analytes were detected in is shown. The Bio-Plex Pro Human Inflammation Assays detected target proteins in 67% more samples than assays from vendor R. Vendor R's 21-plex assay required two base kits and separate runs to accommodate the different sample dilution requirements for certain analytes, while the Bio-Plex Pro 37-Plex Assay required only a single run and single dilution. Bio-Plex Pro Assays (■); vendor R assays (■).

## Product Configuration Options

**Table 2. Comparison of features for each ordering option.**

	Premixed Panels	x-Plex™ Custom Assay (We Mix)	Express Custom Assay (You Mix)	Singleplex/Individual Components
All-in-one kit	•	•	•	
Single-level quality controls	•			
Assay plate type	Flat bottom	Flat bottom	Flat bottom	Flat bottom
Choice of analytes		•	•	•
Number of analytes	37, 24, or 12	11 or higher	10 or lower	10 or lower*
Detailed product data sheet	•	•	•	
Assay quick guide**	•	•	•	•
Faster delivery	•		•	•
Premium performance	•	•		

\* Due to the 10x concentration of detection antibodies in singleplex sets, a maximum of ten individual sets can be mixed by the end user.

\*\* Visit [bio-rad.com/bio-plex](http://bio-rad.com/bio-plex) for assay quick guides and manuals.

## Ordering Information

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

Catalog #	Description
171-AL001M	<b>Bio-Plex Pro Human Inflammation Panel 1, 37-Plex</b> , 1 x 96-well, for the detection of APRIL / TNFSF13, BAFF / TNFSF13B, sCD30 / TNFRSF8, sCD163, chitinase 3-like 1, gp130 / sIL-6R $\beta$ , IFN- $\alpha$ 2, IFN- $\beta$ , IFN- $\gamma$ , IL-2, sIL-6R $\alpha$ , 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- $\lambda$ 2, IL-29 / IFN- $\lambda$ 1, 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
171-AL002M	<b>Bio-Plex Pro Human Inflammation Panel 1, 24-Plex</b> , 1 x 96-well, for the detection of APRIL / TNFSF13, BAFF / TNFSF13B, sCD30 / TNFRSF8, sCD163, chitinase 3-like 1, gp130 / sIL-6R $\beta$ , IFN- $\beta$ , IL-11, IL-19, IL-20, IL-26, IL-27 (p28), IL-28A / IFN- $\lambda$ 2, IL-29 / IFN- $\lambda$ 1, IL-32, IL-34, IL-35, LIGHT / TNFSF14, osteocalcin, pentraxin-3, sTNF-R1, sTNF-R2, TSLP, TWEAK / TNFSF12
171-AL003M	<b>Bio-Plex Pro Human Treg Cytokine Panel 1, 12-Plex</b> , 1 x 96-well, for the detection of IL-2, IL-10, IL-12 (p40), IL-12 (p70), IL-19, IL-20, IL-22, IL-26, IL-27 (p28), IL-28A / IFN- $\lambda$ 2, IL-29 / IFN- $\lambda$ 1, IL-35

### Reagent Kits

171-304090M	<b>Bio-Plex Pro Reagent Kit III</b> , 1 x 96-well, includes detection antibody diluent HB, standard diluent HB, sample diluent HB, assay buffer, wash buffer, streptavidin-PE, flat bottom plate, and sealing tape, for magnetic separation methods
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### Standards

171-DL0001	<b>Bio-Plex Pro Human Inflammation Panel 1 Standard</b> , pkg of 1 vial, lyophilized mixture of 37 standard analytes
171-DL0050	<b>Bio-Plex Pro Human Inflammation Panel 1 Standard</b> , pkg of 50 lot-matched vials, lyophilized mixture of 37 standard analytes

### Wash Stations and Accessories

300-34376	<b>Bio-Plex Pro Wash Station</b> , microplate wash station for magnetic bead-based assays, includes magnetic plate carrier, waste bottle, 2 liquid bottles
171-020100	<b>Bio-Plex Handheld Magnetic Washer</b> , includes magnetic washer and adjustment hex tools for use in manual wash steps for all Bio-Plex magnetic assays
171-025001	<b>Bio-Plex Pro Flat Bottom Plates</b> , pkg of 40, 96-well plates, for use with Bio-Plex Pro Wash Stations when using magnetic bead-based assays

### Software

171-001510	<b>Bio-Plex Data Pro™ Software with Bio-Plex Manager Software</b> , 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
171-001513	<b>Bio-Plex Data Pro Software</b> , (5 seats), for multi-experiment analysis and advanced data visualization
171-STND01	<b>Bio-Plex Manager Software</b> , includes 1 user desktop license, for analysis of Bio-Plex data and generation of protocols, does not operate the instrument with Bio-Plex Pro Wash Stations when using magnetic bead-based assays

### Bio-Plex Pro Human Inflammation Panel Singleplex Sets\*

171-BL001M	APRIL / TNFSF13
171-BL002M	BAFF / TNFSF13B
171-BL003M	sCD30 / TNFRSF8
171-BL004M	sCD163
171-BL005M	Chitinase 3-like 1
171-BL006M	gp130 / sIL-6R $\beta$
171-BL007M	IFN- $\alpha$ 2
171-BL008M	IFN- $\beta$
171-BL009M	IFN- $\gamma$
171-BL010M	IL-2
171-BL011M	sIL-6R $\alpha$
171-BL012M	IL-8
171-BL013M	IL-10
171-BL014M	IL-11
171-BL015M	IL-12 (p40)
171-BL016M	IL-12 (p70)
171-BL017M	IL-19
171-BL018M	IL-20
171-BL019M	IL-22
171-BL020M	IL-26
171-BL021M	IL-27 (p28)
171-BL022M	IL-28A / IFN- $\lambda$ 2
171-BL023M	IL-29 / IFN- $\lambda$ 1
171-BL024M	IL-32
171-BL025M	IL-34
171-BL026M	IL-35
171-BL027M	LIGHT / TNFSF14
171-BL028M	MMP-1
171-BL029M	MMP-2
171-BL030M	MMP-3
171-BL031M	Osteocalcin
171-BL032M	Osteopontin
171-BL033M	Pentraxin-3
171-BL034M	sTNF-R1
171-BL035M	sTNF-R2
171-BL036M	TSLP
171-BL037M	TWEAK / TNFSF12

\* Singleplex sets include coupled magnetic beads and detection antibodies.

Requires Bio-Plex Pro Reagent Kit III and Human Inflammation Panel 1 Standard to run an assay. Up to 10 singleplex assays can be mixed by the end user. Do not mix the Human Inflammation Panel sets with sets from other Bio-Plex panels or groups.

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

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