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

Bio-Plex Pro Human Th17 Cytokine Assays

MAGNETIC SEPARATION ENABLED

 $\text{IL-1}\beta,\text{IL-4},\text{IL-6},\text{IL-10},\text{IL-17A},\text{IL-17F},\text{IL-21},\text{IL-22},\text{IL-23},\text{IL-25},\text{IL-31},\text{IL-33},\text{IFN-}\gamma,\text{sCD40L},\text{TNF-}\alpha,\text{IL-17A/F},\text{IL-17A/F},\text{IL-17A},\text{IL$

- 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 Assays for Immunology Research

The Bio-Plex Pro Human Th17 Cytokine Panel is a unique blend of magnetic bead–based immunoassays designed to meet the needs of the most discerning preclinical and clinical researchers. The multiplex format enables robust and reproducible measurement of 15+1 soluble proteins involved in the T-helper cell type 17 immune response pathway, which is thought to play a key role in inflammatory conditions such as:

- Rheumatoid arthritis, lupus, psoriasis, multiple sclerosis, and other autoimmune diseases
- Antimicrobial immunity
- Transplant rejection
- Cancer

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

- Reagent Kit II with diluents optimized for serum and plasma samples
- Assay quick guide to get you started right away
- Assay protocol optimized for high-precision and broad-assay working ranges
- 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 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 undergo rigorous evaluation that includes the following parameters:

- Specificity and 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 1–3)

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



	A		rking Range, g/ml	Assay Sensitivity, pg/ml	Assay Precision		Calibration to WHO/NIBSC	
Target	Alternate Names	LLOQ	ULOQ	LOD	Intra-Assay %CV	Inter-Assay %CV	Factor**	NIBSC Catalog #
IL-1β	-	0.4	2,877	0.1	1.6	5.4	1.0	86/680
IL-4	-	1.9	7,861	1.9	3.7	10.4	0.7	88/656
IL-6	-	3.1	25,399	2.0	3.1	4.3	1.6	89/548
IL-10	-	3.2	12,923	1.4	3.2	6.0	1.3	93/722
IL-17A	IL-17, CTLA8	1.6	25,915	0.8	3.5	5.1	0.4	01/420
IL-17F	-	7.5	30,743	3.1	3.9	9.8	-	-
IL-21	Za11	15.3	250,446	2.5	3.3	10.3	-	-
IL-22	-	2.5	41,572	0.9	3.3	4.3	-	-
IL-23	IL-23A, SGRF	10.7	132,017	5.2	3.9	6.0	-	-
IL-25	IL-17E	1.3	21,804	0.8	2.6	4.4	-	-
IL-31	-	3.6	58,723	2.1	5.9	10.4	-	-
IL-33	-	6.8	55,852	1.6	2.6	6.1	-	-
IFN-γ	-	0.7	11,377	0.3	3.4	2.1	1.7	87/586
sCD40L	CD154	7.7	126,350	4.4	2.9	3.9	-	-
TNF-α	-	0.3	4,678	0.04	3.2	6.5	0.4	88/786
IL-17A/F*	-	1.62	6,631	1.28	4.3	5.8	-	_

Table 1. Representative assay performance.

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 the mean of eight standard points run in triplicate within one representative assay. LLOQ and ULOQ are defined as the boundary standard curve points that meet precision and accuracy specifications of 10% intra-assay CV and 80–120% recovery. Data were generated using the magnetic workflow with the Bio-Plex Pro II Wash Station.

* IL-17A/F values are derived from the singleplex assay format.

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



Fig. 1. Serum cytokine levels from individuals with psoriasis (PSO, N = 8), rheumatoid arthritis (RA, N = 14), rheumatoid factors (RF, N = 10), and systemic lupus erythematosus (SLE, N = 18). Nonmatching normal (NM, N = 11) serum samples were used as the baseline reference. The *t*-test function in Bio-Plex Data Pro Software was used to calculate statistical differences. Only *P* values with statistical significance are shown.







Fig. 3. sCD40L standard curve with human serum and plasma samples. Data were analyzed using Bio-Plex Manager Software version 6.0. Recovery range specification was set to 80–120%. Standard (\blacksquare); unknown (\triangle); control (\triangle).

Table 2. Comparison of features for each ordering option.

Flexible Ordering Options

For more information see Table 2.

Premixed 15-Plex Panel

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.

Note: IL-17A/F is available only as a singleplex set and cannot be mixed with IL-17A or IL-17F assays.

x-Plex Custom Assays (we mix)

This option is a great choice for premium custommixed assays. Use the online Bio-Plex Assay Builder at **bio-rad.com/assaybuilder** to select your analytes of interest and plate type. Assays are mixed for you at Bio-Rad and delivered as an all-in-one kit.

Express Custom Assays (you mix)

These assays are an economical choice with fast delivery. Simply select up to ten singleplex analytes of interest and plate type using the Bio-Plex Assay Builder. 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. Go to **bio-rad.com/bio-plex** or see the ordering information provided in this bulletin.

Note: The quality controls provided in premixed assay kits are not sold separately.

	Premixed 15-Plex Panel	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	•	•		

* Printed guide included in assay kits. The guide is also available at **bio-rad.com/bio-plex**.

Ordering Information

Catalog# Description Human Th17 Cytokine Assays

Human Th17 Premixed All-In-One Kit

 Bio-Plex Pro Human Th17 Cytokine 15-Plex Panel, 1 x 96-well, includes premixed coupled magnetic beads, detection antibodies, standards, 2-level controls, detection antibody diluent, standard diluent HB, sample diluent HB, assay buffer, wash buffer, streptavidin-phycoerythrin, 96-well flat bottom plate, sealing tape, and instructions for detecting IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN-γ, sCD40L, TNF-α. IL-17A/F not included

Human Th17 Singleplex Sets, 1 x 96-well*

171BA001M	IL-1β	
171BA002M	IL-4	
171BA003M	IL-6	
171BA004M	IL-10	
171BA005M	IL-17A	
171BA006M	IL-17F	
171BA007M	IL-21	
171BA008M	IL-22	
171BA009M	IL-23	
171BA010M	IL-25	
171BA011M	IL-31	
171BA012M	IL-33	
171BA013M	IFN-γ	
171BA014M	sCD40L	
171BA015M	TNF-α	
171BA016M	IL-17A/F**	

Reagent Kit for Use with Human Th17 Assays

171304090M	Bio-Plex Pro Reagent Kit III with Flat Bottom Plate,			
	1 x 96-well, includes detection antibody diluent HB, standard			
	diluent HB, sample diluent HB, assay and wash buffers,			
	streptavidin-phycoerythrin, flat bottom plate, and sealing tape,			
	for magnetic separation methods			

Human Th17 Standards

 171DA0001 Bio-Plex Pro Human Th17 Cytokine Standard, pkg of 1 vial, lyophilized mixture of 16 standard antigens
 171DA0501 Bio-Plex Pro Human Th17 Cytokine Standard, pkg of 50 lot-matched vials, lyophilized mixture of 16 standard antigens

Additional Th17 Pathway Assays

- TGF- β All-In-One Kit
- 171W4001M **Bio-Plex Pro TGF-** β **3-Plex Panel**, 1 x 96-well, includes premixed coupled magnetic beads and detection antibodies, standards, assay buffer, wash buffer, detection antibody diluent, streptavidin-phycoerythrin, flat bottom plate, sealing tape, standard diluent, sample diluent, and instructions for the detection of TGF- β 1, TGF- β 2, and TGF- β 3

TGF- β Singleplex Sets, 1 x 96-well^{***}

171V4001M	TGF-β1
171V4002M	TGF-β2
171V4003M	TGF-β3

	Catalog#	Description				
	TGF-β Standards					
	171X40001	Bio-Plex Pro TGF- β Standard , pkg of 1 vial, TGF- β standard for detecting TGF- β 1, TGF- β 2, and TGF- β 3 analytes				
	171X40501	Bio-Plex Pro TGF- β Standard , pkg of 50 lot-matched vials, TGF- β standard for detecting TGF- β 1, TGF- β 2, and TGF- β 3 analytes				
əll	Reagent Kit fo	r Use with TGF- eta Assays				
9	171304090M	Bio-Plex Pro Reagent Kit III with Flat Bottom Plate , 1 x 96-well, includes detection antibody diluent HB, standard diluent HB, sample diluent HB, assay and wash buffers, streptavidin-phycoerythrin, flat bottom plate, and sealing tape for magnetic separation methods				
	Wash Stations					
	30034376	Bio-Plex Pro Wash Station , includes magnetic plate carrier, waste bottle, 2 buffer bottles				
	Accessories					
	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, 40 × 96-well plates				
	171304500	Bio-Plex Wash Buffer, 1.5 L				
	171304502	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				
	Software					

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			
Bio-Plex Data Pro Software, Bio-Plex Data Pro Software			
(5 seats), for multi-experiment analysis and advanced data			
visualization			
Bio-Plex Manager Software, 1-user desktop license to			
analyze Bio-Plex data and generate protocols, does not			
operate the instrument			
a include acurated magnetic backs and detection antibadias			

- * Singleplex sets include coupled magnetic beads and detection antibodies. Human Th17 Sets also require Bio-Plex Pro Reagent Kit III (171304090M) and Human Th17 Standards to run an assay.
- ** IL-17A/F cannot be mixed with IL-17A or IL-17F Assays.
- *** TGF- β singleplex sets require Bio-Plex Pro Reagent Kit III (171304090M) and TGF- β Standards to run an assay.

Visit bio-rad.com/HumanTh17 for more information.

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19-0451 0719 Sig 0119

