



# Human Th1/Th2 Five-Plex Antibody Bead Kit

## INFORMATION SHEET

Catalog #: LHC0002 Description: Human Th1/Th2 Five-Plex Lot:\* 269868

\*Note: A letter at the end of the lot number signifies an additional packaging of this same lot.

### Intended Use

This kit comprises analyte specific components for the measurement of human IFN- $\gamma$ , IL-2, IL-4, IL-5, and IL-10 in serum, plasma, buffered solution or tissue culture supernatant. The assay may be run alone or in combination with other Antibody Bead Kits from Invitrogen. These reagents are intended for use in the **Luminex® 100™ or 200™ System** only. **This kit is configured for research use only and is not to be used in diagnostic procedures.**

### Reagents Provided

1. Antibody Bead Concentrate (10x):

Catalog #: LM0241 Description: Hu Th1/Th2 Five-Plex Bead (10x) Lot: 270704 Size: 0.25 mL-100 tests

Form: 0.25 mL 10x bead concentrate solution in storage buffer. Contains 7.5 mM sodium azide as preservative.

Storage: **Light-sensitive material.** Store at 2 - 8°C, in the dark, until the expiration date indicated on the kit.

**Recommended Dilution:** If establishing a Five-Plex Assay, see grid below. If establishing a 6-14-Plex Assay, also see the grid below. Use 25  $\mu$ L of reconstituted beads per assay.

2. Biotinylated Antibody Concentrate (10x):

Catalog #: BN0241 Description: Hu Th1/Th2 Five-Plex Biotin (10x) Lot: 270725 Size: 1 mL-100 tests

Form: 1 mL of a 10x stock of Biotinylated Antibody Concentrate in Biotin Diluent. Contains 15 mM sodium azide as preservative. Concentration of antibody is matched to this lot of beads. Do not mix lots of Coated Beads and Detection Antibody.

Storage: Store at 2 - 8°C until the expiration date indicated on the kit.

**Recommended Dilution:** If establishing a Five-Plex Assay, see grid below. If establishing a 6-14-Plex Assay, also see the grid below. Use 100  $\mu$ L of reconstituted biotinylated antibody per assay. See the Assay Protocol for further information.

Reagent Dilution Grid		1 96-well plate	
Multiplex #	Component	Antibody Bead Concentrate	Biotin-Antibody Concentrate
		Volume ( $\mu$ L)	Volume ( $\mu$ L)
5	1st 5-Plex (10x)	250	1000
	Diluent	2500*	10,000**
(6-14)	5-Plex (10x)	250	1000
	each 1-Plex (10x)	250	1000
	Diluent	QS to 2750 $\mu$ L*	QS to 10,100 $\mu$ L**
	Diluted volume added to each well	25 $\mu$ L	100 $\mu$ L
*Working Wash Solution; ** Biotin Diluent			
<b>Note: Store unused multiplexed reagents at 4°C in the dark.</b>			

3. Hu Cytokine Ten-Plex Standard (IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, GM-CSF, TNF- $\alpha$ , and IFN- $\gamma$ ) (2 vials):

Catalog #: SM024 Description: Rec. Hu Cytokine Ten-Plex Std. Lot: 1385190 Size: Single use

Form: Lyophilized. The proteins in this standard have been calibrated against the masses of highly purified recombinant proteins, with the respective Invitrogen ELISA kits, and NIBSC calibration standards (if available). See the Product Insert included in the Buffer Reagent Kit for further information.

Storage: Store at 2 - 8°C. Use within 1 hour after reconstitution. Discard immediately after use.

**Concentration of Reconstituted Standards\*\*:**

IL-2 (8.67 ng/mL)	IL-4 (15.14 ng/mL)	IFN- $\gamma$ (7.58 ng/mL)
IL-5 (8.91 ng/mL)	IL-10 (11.48 ng/mL)	IL-1 $\beta$ (7.67 ng/mL)
IL-8 (14.28 ng/mL)	TNF- $\alpha$ (8.12 ng/mL)	IL-6 (5.14 ng/mL)
GM-CSF (8.79 ng/mL)		

**\*\*Important note: The concentrations of reconstituted standards are lot-specific. Please verify all concentration values entered in data analysis software.**

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PILHC0002 (Rev 09/08)

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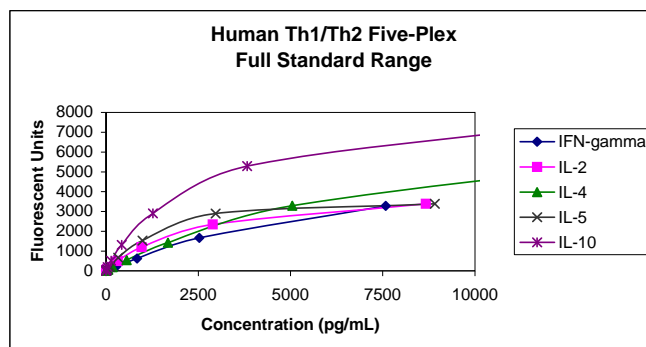
## INFORMATION SHEET

**Reconstitution of Standard:** For a Five-Plex, reconstitute one vial of Human Cytokine Five-Plex Standard in 1.0 mL Assay Diluent, while working with serum or plasma samples. For other sample types, such as tissue culture supernatants, reconstitute one vial of the standard in 1.0 mL of a solution of 50% Assay Diluent + 50% of matrix that matches the sample matrix. Allow standard to rehydrate for at least 10 minutes. If additional standards are to be included with the Human Cytokine Five-Plex panel, reconstitute standards so that the total reconstitution volume (of all standards) does not exceed 1 mL (e.g., if three 3 standards are to be used, reconstitute each in 0.33 mL).

**Recommended Starting Concentration for Standard Curve:** Upon reconstitution, the starting concentrations of the standard are the values cited above. Make serial 1:3 dilutions in the same diluent used to reconstitute the standard. Use 100 µL per assay. See the included Assay Protocol for further information.

### Performance Characteristics

Typical Standard Curve



Cytokine	IFN- $\gamma$	IL-2	IL-4	IL-5	IL-10
Bead Region	38	54	77	34	9
Sensitivity (pg/mL)	5	6	5	3	5
Recovery (Serum)	105%	111%	94%	83%	103%
Recovery (TC)	100%	100%	100%	100%	100%
Recovery (Plasma)	107%	111%	86%	96%	85%
NIBSC calibration	1ng= 20 IU	1ng= 13 IU	1ng= 10 IU	1ng= 10 IU	1ng= 5 IU
Inter assay variation	9%	9.5%	8.7%	7.5%	9.8%
Invitrogen ELISA (Catalog #)	KAC1231	KAC1241	KAC1281	KHC0051	KAC1321
Correlation coefficient	0.98	0.94	0.99	0.99	0.93
(Multiplication factor) *	1.12	0.52	1.5	0.47	0.99

**EQUATION:** Bead kit value x \*Multiplication Factor = ELISA value

While the diluents included in the kits for determinations of the individual cytokines were developed to optimize quantitation and recovery, the Five-Plex assay requires the use of a common diluent. In order to determine the impact of the common diluent on quantitation, correlation studies were performed using stimulated lymphocytes in RPMI + 10% FCS. Data described here were generated with the designated Invitrogen ELISA kit. Correlation of results obtained with the Five-Plex Antibody Bead kit to one's own system should be determined to arrive at an appropriate multiplication factor.

**Specificity:** The analytes of the multiplex were assayed individually at 10 or 20 ng/mL with the Invitrogen Human Th1/Th2 Five-Plex Antibody Bead Kit and all were found to have no cross-reactivity.

By purchasing this Kit, which contains fluorescently labeled microsphere beads authorized by Luminex® Corporation ("Luminex®"), you, the customer, acquire the right under Luminex's patent rights to use this Kit or any portion of this Kit, including without limitation the microsphere beads contained herein, only with Luminex's laser based fluorescent analytical test instrumentation marketed under the name Luminex® 100™ or 200™. This product is covered by one or more of the following U.S. patents: 6,046,807.

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