



Human Cytokine Magnetic 25-Plex Panel

Technical Data Sheet

Catalog #: LHC0009M Lot #:* 910513

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

Intended Use

This kit is comprised of components for the measurement of human IL-1 β , IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40/p70, IL-13, IL-15, IL-17, TNF- α , IFN- α , IFN- γ , GM-CSF, MIP-1 α , MIP-1 β , IP-10, MIG, Eotaxin, RANTES, and MCP-1 in serum, plasma, or tissue culture supernatant. The assay may be run alone or in combination with other Singleplex Bead Kits from Invitrogen. These reagents are intended for use in the **Luminex® 100™/200™ and FlexMAP 3D® System** only. **This kit is configured for research use only and is not to be used in diagnostic procedures.** Recommended Sample Types: For best results with this 25-Plex Panel, serum and tissue culture medium samples are recommended. See pages 3 and 4 for Recovery results on serum, tissue culture medium, and plasma samples.

Reagents Provided

- | | | | | | | | | |
|----|----------|--|--------------|---|------|--------|-------|------------------|
| 1. | Part #: | LM317 | Description: | Hu Cytokine Mag 25-Plex Antibody Bead Solution (1X) | Lot: | 910514 | Size: | 2.5 mL-100 tests |
| | Form: | 2.5 mL 1X bead solution in storage buffer. Contains 0.05% sodium azide as a preservative. | | | | | | |
| | Storage: | Light-sensitive material. Store at 2 to 8°C, in the dark, until the expiration date indicated on the kit. | | | | | | |
| 2. | Part #: | BN317 | Description: | Hu Cytokine Mag 25-Plex Biotinylated Ab Conc. (10X) | Lot: | 910515 | Size: | 1 mL-100 tests |
| | Form: | 1 mL of a 10X stock of Biotinylated Antibody Concentrate in Biotin Diluent. Contains 0.1% sodium azide as preservative. Concentration of antibody is matched to this lot of beads. Do not mix lots of Coated Beads and Biotinylated Antibody. | | | | | | |
| | Storage: | Store at 2 to 8°C until the expiration date indicated on the kit. | | | | | | |
| 3. | Part #: | SM310 | Description: | Hu 14-Plex Standard | Lot: | 836090 | Size: | 2 Vials |
| | 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). Please see the table presented on page 3 for further information. Contains 0.1% sodium azide as a preservative. | | | | | | |
| | Storage: | Store at 2 to 8°C. Use within 1 hour after reconstitution. Discard immediately after use. | | | | | | |

Concentration of Reconstituted Standards**:

VEGF (4,400 pg/mL)	G-CSF (70,000 pg/mL)	EGF (12,000 pg/mL)	HGF (7,900 pg/mL)
FGFbasic (2,400 pg/mL)	RANTES (3,500 pg/mL)	Eotaxin (2,900 pg/mL)	MIP-1 α (25,000 pg/mL)
MIP-1 β (7,200 pg/mL)	MCP-1 (6,100 pg/mL)	IL-1RA (28,000 pg/mL)	IP-10 (900 pg/mL)
IL-2R (21,000 pg/mL)	MIG (2,400 pg/mL)		

- | | | | | | | | | |
|----|----------|---|--------------|---------------------|------|--------|-------|---------|
| 4. | Part #: | SM311 | Description: | Hu 16-Plex Standard | Lot: | 891415 | Size: | 2 Vials |
| | 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. Contains 0.1% sodium azide as a preservative. | | | | | | |
| | Storage: | Store at 2 to 8°C. Use within 1 hour after reconstitution. Discard immediately after use. | | | | | | |

Concentration of Reconstituted Standards**:

IL-1 β (8,300 pg/mL)	IL-10 (21,900 pg/mL)	IFN- α (12,900 pg/mL)	IL-6 (4,150 pg/mL)
IL-12 (6,500 pg/mL)	IL-13 (20,300 pg/mL)	IL-15 (24,300 pg/mL)	IL-17 (38,800 pg/mL)
GM-CSF (17,350 pg/mL)	IL-5 (2,330 pg/mL)	IFN- γ (10,500 pg/mL)	TNF- α (8,700 pg/mL)
IL-2 (1,200 pg/mL)	IL-7 (9,800 pg/mL)	IL-4 (42,600 pg/mL)	IL-8 (12,800 pg/mL)

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

Reconstitution of Standard: Each 25-Plex standard curve requires one vial each of Hu 14-Plex Standard and Hu 16-Plex Standard. Each standard vial must be reconstituted separately with 0.5 mL and allowed to rehydrate for at least 10 minutes. The contents of each of the two vials should then be combined for a total volume of 1.0 mL. When working with serum or plasma, reconstitute each vial with 0.5 mL Assay Diluent, allow to rehydrate, mix gently, and combine for a total volume of 1.0 mL. When working with other sample types, such as tissue culture supernatant, reconstitute each vial with 0.5 mL of a solution composed of 50% Assay Diluent + 50% of matrix that matches the sample matrix, allow to rehydrate, and combine for a total volume of 1.0 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 μ L per assay. Refer to the User Manual for further information.

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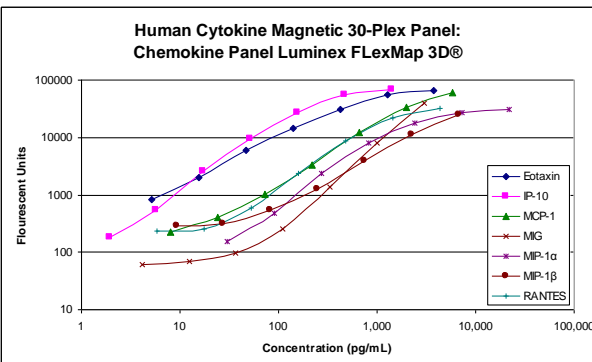
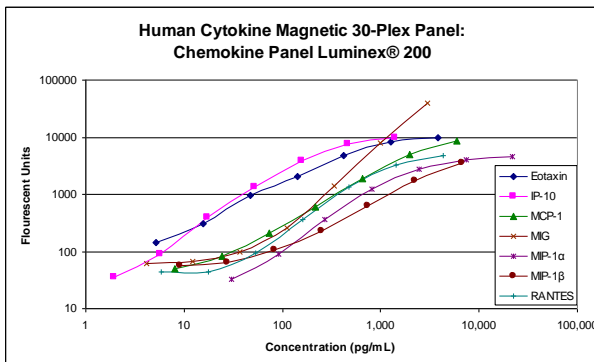
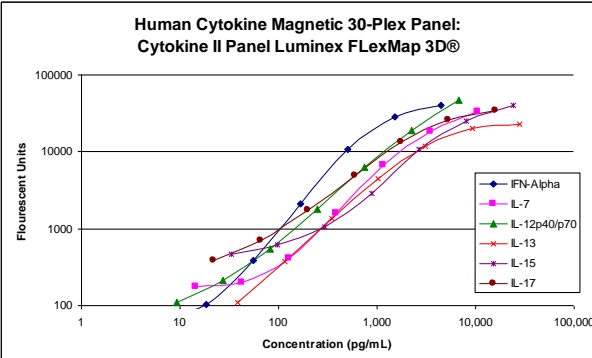
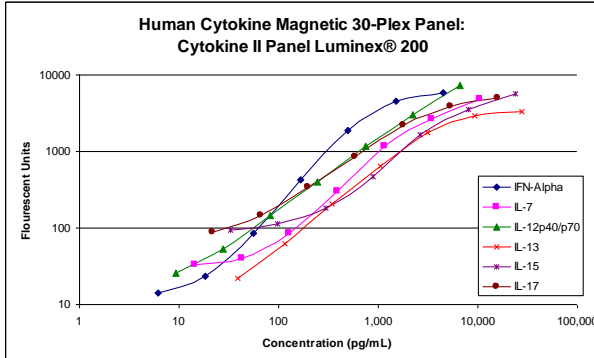
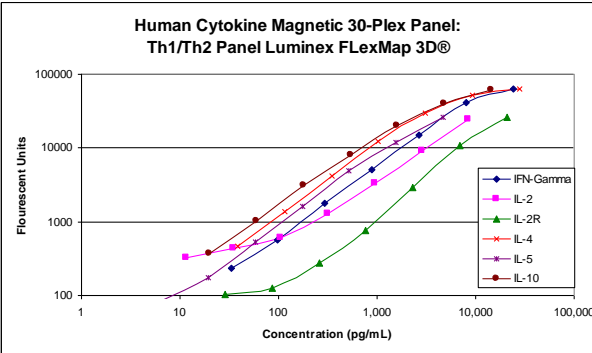
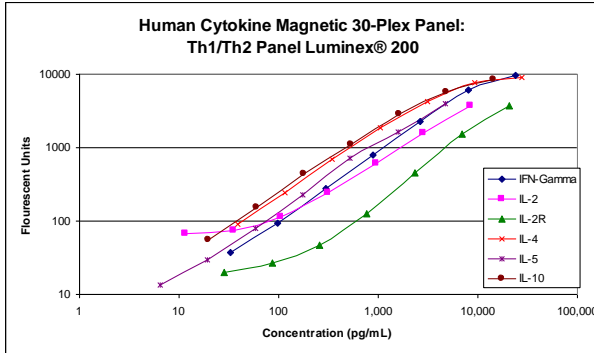
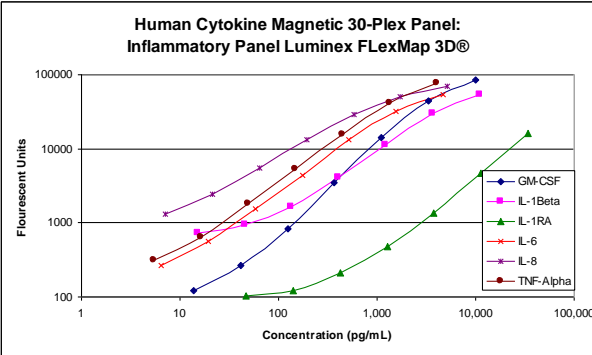
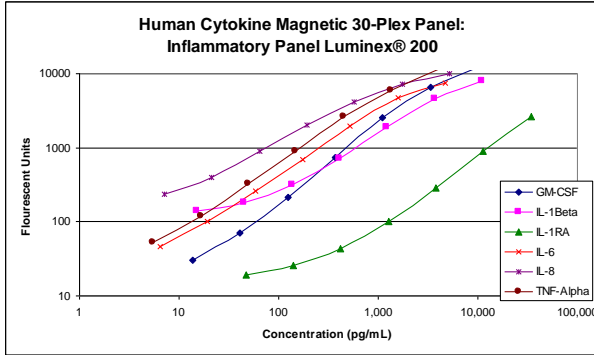
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Performance Characteristics

Typical Standard Curves: The data presented here were collected as a 25-Plex, but are presented in four groupings for clarity.



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Performance Characteristics

Cytokine	IL-1 β	IL-10	IFN- α	IL-6	IL-12
Bead Region	13	15	43	19	20
Sensitivity (pg/mL)	<15	<5	<20	<3	<15
Recovery (Serum)	80% - 120%	80% - 120%	<70%	80% - 120%	80% - 120%
Recovery (EDTA Plasma)	80% - 120%	80% - 120%	<70%	80% - 120%	80% - 120%
Recovery (Citrate Plasma)	80% - 120%	80% - 120%	80% - 120%	80% - 120%	80% - 120%
Recovery (Heparin Plasma)	80% - 120%	80% - 120%	70% - 130%	80% - 120%	Not Recommended
Recovery (TC) RPMI+10% FBS	80% - 120%	80% - 120%	80% - 120%	80% - 120%	80% - 120%
NIBSC calibration	1 ng = 25 IU	1 ng = 13 IU	1 ng = 14 IU	1 ng = 30 IU	1 ng = 11 IU
NIBSC Preparation Number	86 / 680	93 / 722	94 / 784	89 / 548	95 / 544
Intra assay variation	6%	7%	3%	3%	3%
Invitrogen ELISA (Catalog #)	KHC0011	KHC0101	KHC4012	KHC0061	KHC0121
Multiplication factor [†]	3.2	0.6	6.7	2.1	1.5

Cytokine	RANTES	Eotaxin	IL-13	IL-15	IL-17
Bead Region	21	22	18	30	25
Sensitivity (pg/mL)	<15	<5	<10	<30	<16
Recovery (Serum)	***	70% - 130%	80% - 120%	80% - 120%	<70%
Recovery (EDTA Plasma)	***	70% - 130%	80% - 120%	80% - 120%	<70%
Recovery (Citrate Plasma)	***	80% - 120%	80% - 120%	80% - 120%	70% - 130%
Recovery (Heparin Plasma)	***	Not Recommended	80% - 120%	80% - 120%	Not Recommended
Recovery (TC) RPMI+10% FBS	80% - 120%	80% - 120%	80% - 120%	80% - 120%	80% - 120%
NIBSC calibration	1 ng = 0.7 IU	NA	1 ng = 1.2 IU	1 ng = 150 IU	1 ng = 10 IU
NIBSC Preparation Number	92 / 520	NA	94 / 622	95 / 554	01 / 420
Intra assay variation	3%	10%	5%	2%	4%
Invitrogen ELISA (Catalog #)	KHC1031	NA	KHC0131	KHC0151	NA
Multiplication factor [†]	1.4	4.2	0.83	0.2	2.0

***Additional sample dilution required for accurate recovery

Cytokine	MIP-1 α	GM-CSF	MIP-1 β	MCP-1	IL-5
Bead Region	26	27	28	29	34
Sensitivity (pg/mL)	<10	<15	<10	<10	<3
Recovery (Serum)	80% - 120%	80% - 120%	80% - 120%	70% - 130%	80% - 120%
Recovery (EDTA Plasma)	80% - 120%	70% - 130%	80% - 120%	>130%	80% - 120%
Recovery (Citrate Plasma)	80% - 120%	80% - 120%	70% - 130%	>130%	<70%
Recovery (Heparin Plasma)	80% - 120%	80% - 120%	<70%	80% - 120%	70% - 130%
Recovery (TC) RPMI+10% FBS	80% - 120%	80% - 120%	80% - 120%	80% - 120%	80% - 120%
NIBSC calibration	1 ng = 0.4 IU	1 ng = 6 IU	NA	1 ng = 1.5 IU	1 ng = 0.2 IU
NIBSC Preparation Number	92 / 518	88 / 646	NA	92 / 794	90 / 586
Intra assay variation	3%	3%	5%	8%	4%
Invitrogen ELISA (Catalog #)	KAC2201	KHC2011	KAC2291	KHC1011	KHC0051
Multiplication factor [†]	0.47	2.4	0.6	0.65	4.4

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Cytokine	IFN- γ	TNF- α	IL-1RA	IL-2	IL-7
Bead Region	38	52	51	54	55
Sensitivity (pg/mL)	<5	<10	<30	<6	<16
Recovery (Serum)	80% - 120%	80% - 120%	70% - 130%	80% - 120%	80% - 120%
Recovery (EDTA Plasma)	80% - 120%	80% - 120%	80% - 120%	80% - 120%	80% - 120%
Recovery (Citrate Plasma)	80% - 120%	80% - 120%	70% - 130%	80% - 120%	<70%
Recovery (Heparin Plasma)	Not Recommended	80% - 120%	80% - 120%	70% - 130%	Not Recommended
Recovery (TC) RPMI+10% FBS	80% - 120%	80% - 120%	80% - 120%	80% - 120%	80% - 120%
NIBSC calibration	1 ng = 0.7 IU	1 ng = 0.2 IU	1 ng = 0.001 IU	1 ng = 7 IU	1 ng = 64 IU
NIBSC Preparation Number	82 / 587	88 / 786	92 / 644	86 / 564	90 / 530
Intra assay variation	4%	6%	3%	6%	5%
Invitrogen ELISA (Catalog #)	KHC4021	KHC3011	KAC1181	KHC0021	KHC0021
Multiplication factor†	4.5	2.3	1.1	1.5	0.86

Cytokine	IP-10	IL-2R	MIG	IL-4	IL-8
Bead Region	56	61	63	77	78
Sensitivity (pg/mL)	<5	<60	<45	<5	<3
Recovery (Serum)	80% - 120%	80% - 120%	80% - 120%	80% - 120%	80% - 120%
Recovery (EDTA Plasma)	<70%	80% - 120%	80% - 120%	80% - 120%	80% - 120%
Recovery (Citrate Plasma)	80% - 120%	80% - 120%	>130%	80% - 120%	80% - 120%
Recovery (Heparin Plasma)	Not Recommended	80% - 120%	Not Recommended	70% - 130%	80% - 120%
Recovery (TC) RPMI+10% FBS	80% - 120%	80% - 120%	80% - 120%	80% - 120%	80% - 120%
NIBSC calibration	NA	1 ng = 12.2–40.6 IU	NA	1 ng = 65 IU	1 ng = 40 IU
NIBSC Preparation Number	NA	97 / 600	NA	88 / 656	89 / 520
Intra assay variation	10%	4%	10%	3%	3%
Invitrogen ELISA (Catalog #)	KHC0071	KHR0021	KAC2431	KHC0041	KHC0081
Multiplication factor†	0.68	1.3	1.15	0.26	0.15

EQUATION: (Human Cytokine 25-Plex Panel 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 25-Plex assay requires the use of a common diluent. Correlation of results obtained with the Human Cytokine 25-Plex Panel to one's own system should be determined to arrive at an appropriate multiplication factor.

Specificity: The analytes of the multiplex were assayed individually with the Human Cytokine 25-Plex Panel. All were found to have cross-reactivity of < 1%.

Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		In vitro diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
	Without, does not contain		With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

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