

**\*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 mouse IL-2, IL-4, IL-5, IL-10, IL-12, and IFN- $\gamma$  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™ or 200™ System** only. **This kit is configured for research use only and is not to be used in diagnostic procedures.**

Please note that the buffers included in this Mouse Th1/Th2 6-Plex Panel are not compatible with the Invitrogen Mouse G-CSF Singleplex Bead Kit (Catalog# LMC2011) or the Mouse PDGF-BB Singleplex Bead Kit (Catalog# LMG0041). When developing multiplexed assays with mouse cytokine and growth factor Singleplex Bead Kits, Catalog# LMB0002 should be used as the common buffer kit.

### Reagents Provided

- |                 |  |  |   |
|-----------------|--|--|---|
|                 |  | Ms Th1/Th2 6-Plex  |   |
| 1.              | <b>Part #:</b> <u>LM0431</u>   | <b>Description:</b> <u>Antibody Bead Concentrate (10X)</u> | <b>Lot:</b> <u>719602</u> <b>Size:</b> <u>0.25 mL-100 tests</u> |
| <b>Form:</b>    | 0.25 mL 10X bead concentrate solution in storage buffer. Contains 0.05% sodium azide as a preservative.  |  |   |
| <b>Storage:</b> | <b>Light-sensitive material.</b> Store at 2 to 8°C, in the dark, until the expiration date indicated on the kit.   |  |   |
|                 |  | Ms Th1/Th2 6-Plex  |   |
| 2.              | <b>Part #:</b> <u>BN0431</u>   | <b>Description:</b> <u>Biotinylated Ab Conc. (10X)</u>     | <b>Lot:</b> <u>719601</u> <b>Size:</b> <u>1 mL-100 tests</u>    |
| <b>Form:</b>    | 1 mL of a 10X stock of Biotinylated Antibody Concentrate in Biotin Diluent. Contains 0.1% sodium azide. Concentration of antibody is matched to this lot of beads. Do not mix lots of Coated Beads and Detection Antibody.   |  |   |
| <b>Storage:</b> | Store at 2 to 8°C until the expiration date indicated on the kit.  |  |   |
|                 |  | Ms 20-Plex Standard  |   |
| 3.              | <b>Part #:</b> <u>SM039</u>  | <b>Description:</b> <u>Ms 20-Plex Standard</u>             | <b>Lot:</b> <u>675247</u> <b>Size:</b> <u>2 Vials</u>           |
| <b>Form:</b>    | 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). Contains 0.1% sodium azide as a preservative. |  |   |
| <b>Storage:</b> | Store at 2 to 8°C. Use within 1 hour after reconstitution. Discard remaining reconstituted standard immediately after use.   |  |   |

### Concentration of Reconstituted Standards:

FGF basic (51,400 pg/mL)	GM-CSF (8,300 pg/mL)	IFN- $\gamma$ (12,700 pg/mL)	IL-1 $\alpha$ (20,900 pg/mL)
IL-1 $\beta$ (17,000 pg/mL)	IL-2 (8,700 pg/mL)	IL-4 (22,500 pg/mL)	IL-5 (13,800 pg/mL)
IL-6 (15,600 pg/mL)	IL-10 (10,600 pg/mL)	IL-12 (5,000 pg/mL)	IL-13 (16,800 pg/mL)
IL-17 (2,580 pg/mL)	IP-10 (14,000 pg/mL)	KC (140,000 pg/mL)	MCP-1 (18,200 pg/mL)
MIG (3,500 pg/mL)	MIP-1 $\alpha$ (13,000 pg/mL)	TNF- $\alpha$ (12,700 pg/mL)	VEGF (5,700 pg/mL)

**Note: During calibration, the protein values required adjustment; the new values have been designated in the table above. If comparing data from previous lot, refer to Appendix A for Multiplication Factor. Please contact Tech Support if further information is required.**

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

**Reconstitution of Standard:** When working with serum samples, reconstitute one vial of Ms 20-Plex Standard in 1.0 mL Assay Diluent. For other sample types, such as tissue culture supernatants, reconstitute one vial of Ms 20-Plex Standard with 1.0 mL of a solution composed of 50% Assay Diluent + 50% of matrix that matches the sample matrix. Allow the standard to rehydrate for at least 10 minutes, gently vortex, then proceed with further dilution.

**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  $\mu$ L per assay. Refer to User Manual for further information.

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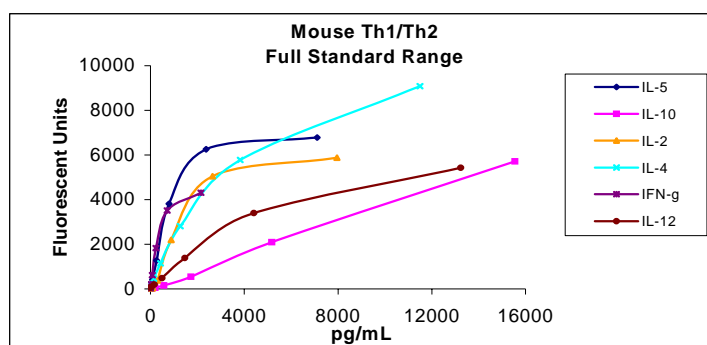
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# Mouse Th1/Th2 6-Plex Panel

## Technical Data Sheet

### Performance Characteristics

#### Typical Standard Curve



Cytokine	Ms IL-2	Ms IL-4	Ms IL-5	Ms IL-10	Ms IL-12	Ms IFN- $\gamma$
Bead Region	54	77	34	9	20	38
Sensitivity (pg/mL)	<20	<15	<10	<15	<15	<2
Recovery (Serum)	97%	73%	101%	104%	83%	109%
Recovery (TC) RPMI+10% FBS	100%	100%	100%	100%	100%	100%
Recovery (EDTA Plasma)	Not recommended	83%	100%	97%	71%	110%
Recovery (Citrate Plasma)	88%	94%	103%	108%	72%	Not recommended
Recovery (Heparin Plasma)	87%	81%	102%	94%	Not recommended	Not recommended
NIBSC calibration	1 ng=100 IU	1ng=10 IU	NA	NA	NA	NA
Inter assay variation	9.2%	8.7%	9.1%	6.5%	8.7%	9.9%
Invitrogen ELISA (Catalog #)	KMC0021	KMC0041	KMC0051	KMC0101	KMC0121	N/A
Correlation coefficient	0.94	0.95	0.98	0.9	0.99	N/A
(Multiplication factor) *	1.0	0.8	1.0	1.0	1.0	N/A

**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 Mouse 6-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 either using stimulated mouse splenocytes in RPMI + 10% FBS or recombinant protein spiked into RPMI + 10% FBS. Data described here were generated with the designated Invitrogen ELISA kit. Correlation of results obtained with the Mouse Th1/Th2 6-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 at 10 or 20 ng/mL with the Invitrogen Mouse Th1/Th2 6-Plex Panel 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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