

**Recommended Sample Types**

- **Human HS Cytokine Base Kit A:** Serum, EDTA plasma, and heparin plasma.
- **Human HS Cytokine Base Kit B:** Cell culture supernates, serum, EDTA plasma, and heparin plasma.

**Microparticle Region**

- Region-25

**Components**

- Microparticle Concentrate (Part 894494) is supplied as a 50X concentrated stock (0.075 mL) with preservatives.
- Biotin-Antibody Concentrate (Part 894051) is supplied as a 100X concentrated stock solution (0.075 mL) with preservatives.

**Other Supplies Required**

- Magnetic Luminex® Performance Assay Human High Sensitivity Cytokine Base Kit A (R&D Systems®, Catalog # LHSCM000) or Magnetic Luminex® Performance Assay Human High Sensitivity Cytokine Base Kit B (R&D Systems®, Catalog # LBHS000).

**Storage**

- Store the unopened kit at 2-8 °C. Do not use past the expiration date on the label.
- **Avoid freezing microparticles.**

**Instructions for Use**

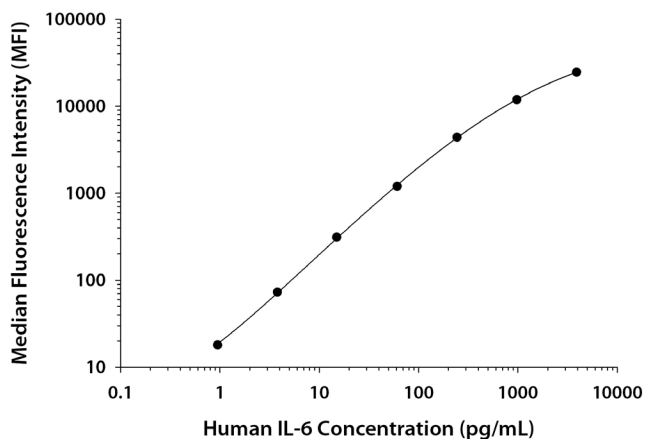
- **Protect microparticles from light.**
- Refer to the appropriate Base Kit insert for the Magnetic Luminex® Performance Assay procedure.

**TYPICAL DATA**

This human IL-6 standard curve is provided only for demonstration. A standard curve must be generated each time an assay is run, utilizing values from the Standard Value Card included in the Base Kit.

**Human HS Cytokine Base Kit A:** When using Calibrator Diluent RD6-40, a seven point standard curve (0.95-3900 pg/mL) is recommended.

**Human HS Cytokine Base Kit B:** When running cell culture supernate samples using Calibrator Diluent RD5K, a six-point standard curve (0.647-663 pg/mL) is recommended. When running serum/plasma samples using Calibrator Diluent RD6-65, a seven-point standard curve (0.647-2650 pg/mL) is recommended.



Standard	(pg/mL)	MFI	Average	Corrected
Blank	0	19 20	20	—
1	3900	24,526 24,710	24,618	24,598
2	975	11,811 12,004	11,908	11,888
3	244	4406 4417	4412	4392
4	61	1220 1220	1220	1200
5	15	327 336	332	312
6	3.8	92 93	92	72
7	0.95	37 38	38	18

**PERFORMANCE CHARACTERISTICS**

**All data were collected with assays run as a multiplex.**

**Data obtained with polystyrene and magnetic beads were equivalent.**

Twenty-eight assays were evaluated, and the minimum detectable dose (MDD) of human IL-6 ranged from 0.08-0.31 pg/mL. The mean MDD was 0.14 pg/mL.

The MDD was determined by adding two standard deviations to the MFI of twenty zero standard replicates and calculating the corresponding concentration.

## PRECISION

**Intra-assay Precision** (precision within an assay) - Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

**Inter-assay Precision** (precision between assays) - Three samples of known concentration were tested in sixty separate assays to assess inter-assay precision.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	60	60	60
Mean (pg/mL)	8.3	58	1217	7.6	57	1111
Standard deviation	0.5	2.1	73	0.8	5.1	103
CV (%)	6.0	3.6	6.0	10.5	8.9	9.3

## RECOVERY

Samples containing and/or spiked with human IL-6 were evaluated for recovery.

Sample Type	Average % Recovery	Range
Cell culture supernates	100	94-104%
Serum	100	83-125%
EDTA plasma	98	80-121%
Heparin plasma	104	86-121%

## LINEARITY

Samples containing and/or spiked with human IL-6 were serially diluted to evaluate assay linearity.

		Cell culture supernates*	Serum	EDTA plasma	Heparin plasma
1:2	Average % of Expected	101	109	105	99
	Range (%)	99-103	105-115	92-111	96-103
1:4	Average % of Expected	104	114	107	97
	Range (%)	101-106	103-133	89-120	83-112
1:8	Average % of Expected	103	116	107	99
	Range (%)	102-139	105-139	87-128	88-116

\*Cell culture supernates are valid samples in Human HS Cytokine Base Kit B only.

## SPECIFICITY

**Note:** Refer to the base kit insert for a complete list of analytes tested for cross-reactivity and interference.

This assay recognizes natural and recombinant human IL-6.

## TECHNICAL HINTS

- Protect the microparticles and streptavidin-PE from light at all times.
- Refer to the Base Kit Standard Value Card for reconstitution volume and values of the reconstituted standard.
- Diluted microparticles cannot be stored. Make a fresh dilution of microparticles each time the assay is run.
- The use of a magnetic device made to accommodate a microplate is necessary for washing.
- Discrepancies may exist in values obtained for the same analyte utilizing different technologies.

Magnetic Luminex® Performance Assays afford the user the benefit of multi-analyte analysis of cytokines in a complex sample. A single, multipurpose diluent for each sample type is used to optimize recovery, linearity, and reproducibility. Such a multipurpose, single diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions. Therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.

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