

**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-12

**Components**

- Microparticle Concentrate (Part 894500) is supplied as a 50X concentrated stock (0.075 mL) with preservatives.
- Biotin-Antibody Concentrate (Part 894057) 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**

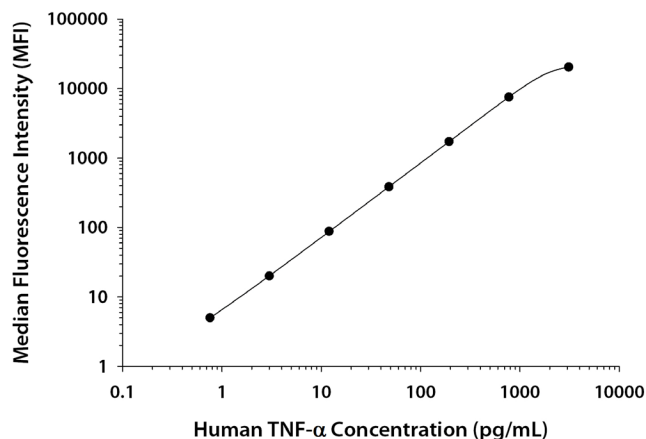
- **Protect microparticles from light.**
- Refer to the appropriate Base Kit insert for the Magnetic Luminex® Performance Assay procedure.

**TYPICAL DATA**

This human TNF-α 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 (0.76-3100 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 (1.20-1230 pg/mL) is recommended. When running serum/plasma samples using Calibrator Diluent RD6-65, a seven-point standard curve (1.20-4920 pg/mL) is recommended.



Standard	(pg/mL)	MFI	Average	Corrected
Blank	0	15 16	16	—
1	3100	20,284 20,416	20,350	20,334
2	775	7375 7725	7550	7534
3	194	1722 1756	1739	1723
4	48	397 400	398	382
5	12	102 106	104	88
6	3	35 36	36	20
7	0.76	20 21	20	4

**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 TNF-α ranged from 0.13-0.54 pg/mL. The mean MDD was 0.29 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)	8.1	65	1533	7.5	61	1654
Standard deviation	0.4	2.7	105	0.8	4.9	169
CV (%)	4.9	4.1	6.8	10.7	8.0	10.2

## RECOVERY

Samples containing and/or spiked with human TNF- $\alpha$  were evaluated for recovery.

Sample Type	Average % Recovery	Range
Cell culture supernates	105	99-111%
Serum	86	73-101%
EDTA plasma	79	73-87%
Heparin plasma	79	70-94%

## LINEARITY

Samples containing and/or spiked with human TNF- $\alpha$  were serially diluted to evaluate assay linearity.

		Cell culture supernates*	Serum	EDTA plasma	Heparin plasma
1:2	Average % of Expected	98	108	111	112
	Range (%)	95-101	98-116	107-115	110-113
1:4	Average % of Expected	94	112	115	111
	Range (%)	93-96	105-125	97-128	107-117
1:8	Average % of Expected	93	116	114	116
	Range (%)	90-97	105-139	99-129	110-123

\*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 TNF- $\alpha$ .

Recombinant human TNF RI interferes at concentrations > 25.0 ng/mL in this assay.

## 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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