Magnetic Luminex® Performance Assay
Human VEGF High Sensitivity Kit

Catalog Number: LHSCM293
Pack Size: 100 Tests

SPECIFICATIONS AND USE

Recommended Sample Types
- Serum, EDTA plasma, and heparin plasma.

Microparticle Region
- Region-13

Components
- Microparticle Concentrate (Part 894501) is supplied as a 50X concentrated stock (0.075 mL) with preservatives.
- Biotin-Antibody Concentrate (Part 894058) is supplied as a 100X concentrated stock solution (0.075 mL) with preservatives.

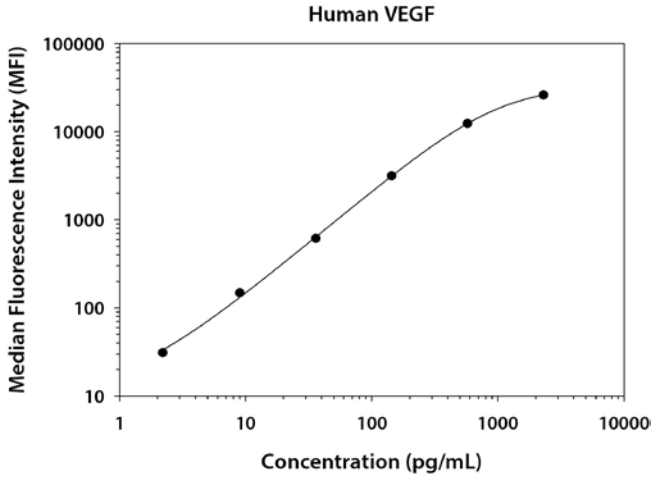
Other Supplies Required
- Magnetic Luminex Performance Assay Human High Sensitivity Cytokine Base Kit A (Catalog Number LHSCM000).

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

Instructions for Use
- Refer to the Base Kit insert for the Magnetic Luminex Performance Assay procedure.

TYPICAL DATA

This human VEGF 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.



Standard	pg/mL	MFI	Average	Corrected
Blank	0	95 97	96	——
1	2300	26,053 26,358	26,206	26,110
2	575	12,124 12,822	12,473	12,377
3	144	3207 3293	3250	3154
4	36	708 712	710	614
5	9	242 246	244	148
6	2.2	125 128	127	31

R&D Systems, Inc.
1-800-343-7475

PRECISION

Intra-assay Precision (precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess precision within an assay.
Inter-assay Precision (precision between assays)
Three samples of known concentration were tested in separate assays to assess precision between assays.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	60	60	60
Mean (pg/mL)	5.6	37	575	4.4	33	590
Standard Deviation	0.7	1.7	36	0.7	4.3	64
% CV	12.5	4.6	6.3	15.9	13.0	10.8

RECOVERY & LINEARITY

Samples were spiked with human VEGF and evaluated for recovery and were serially diluted to evaluate assay linearity.

Recovery			Linearity				
Sample Type	Average % Recovery	Range (%)			Serum	EDTA Plasma	Heparin Plasma
Serum	84	73-115	1:2	Average % of Expected	114	105	114
				Range (%)	103-124	101-110	111-119
EDTA plasma	78	68-87	1:4	Average % of Expected	121	112	119
				Range (%)	119-125	101-124	113-123
Heparin plasma	76	59-94	1:8	Average % of Expected	123	111	120
				Range (%)	113-128	104-122	109-132

SENSITIVITY

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 VEGF ranged from 0.47-1.35 pg/mL. The mean MDD was 0.88 pg/mL.

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

CORRELATION

This assay has been correlated to the Quantikine® ELISA Kit with a slope of 0.9-1.1 and an R^2 value greater than 0.9.

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 VEGF

The factors listed below cross-react as noted:

Mouse $VEGF_{164}$	Human VEGF-D	Human $VEGF_{121}$	Human $VEGF_{206}$
1.3%	1.2%	52.0%	1.1%

TECHNICAL HINTS

- Protect the microparticles and streptavidin-PE from light at all times.
- Refer to the appropriate 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.

Luminex Performance Assays afford the user the benefit of multianalyte analysis of cytokines in a complex sample. A single, multipurpose diluent 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.