Magnetic Luminex® Performance Assay Human Endostatin Kit

Catalog Number: LANM1098
Pack Size: 100 Tests

SPECIFICATIONS AND USE

Recommended Sample Types
- Cell culture supernates, serum, EDTA plasma, heparin plasma, urine, and human milk.

Microparticle Region
- Region-14

Components
- Microparticle Concentrate (Part 894454) is supplied as a 100X concentrated stock (0.075 mL) with preservatives.
- Biotin-Antibody Concentrate (Part 893618) is supplied as a 100X concentrated stock solution (0.075 mL) with preservatives.

Other Supplies Required
- Magnetic Luminex Performance Assay Human Angiogenesis Base Kit A (Catalog Number LANM000).

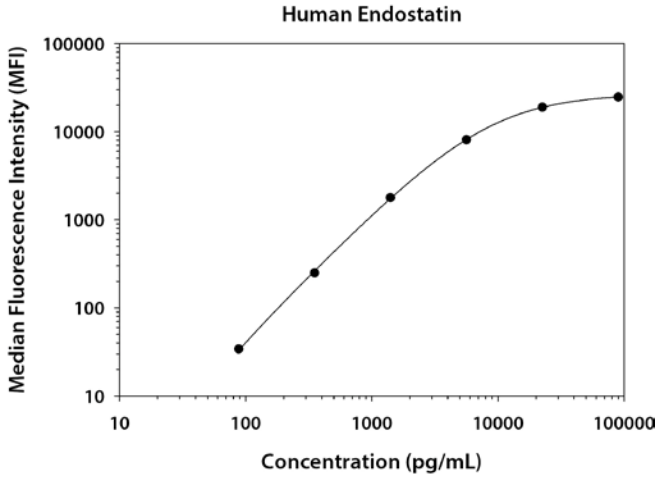
Storage
- Store the unopened kit at 2-8 °C. Do not use past the expiration date on the label.
- **Avoid freezing microparticles.**
- **Protect microparticles from light.**

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

TYPICAL DATA

This human Endostatin 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.

Note: *This kit utilizes a six point standard curve. When fitting a standard curve constructed with the recommended 3-fold dilution series, only use the first six points (omit the lowest concentration standard).*



Standard	pg/mL	MFI	Average	Corrected
Blank	0	14 14	14	——
1	90,000	24,506 25,033	24,770	24,756
2	22,500	18,887 19,108	18,998	18,984
3	5625	8109 8127	8118	8104
4	1406	1794 1799	1797	1783
5	352	261 264	263	249
6	88	47 48	48	34

PERFORMANCE CHARACTERISTICS

All data were collected with assays run as a multiplex.
Data obtained with polystyrene and magnetic beads were equivalent.

Sensitivity - The Minimum Detectable Dose (MDD) was determined by adding two standard deviations to the mean MFI of twenty zero standard replicates and calculating the corresponding concentration.

Thirty assays were evaluated, and the MDD of human Endostatin ranged from 4.3-31.9 pg/mL. The mean MDD was 12.8 pg/mL.

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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 fifty-two separate assays to assess precision between assays.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	52	52	52
Mean (pg/mL)	148	550	9065	158	623	9822
Standard Deviation	15.1	31.4	862	23.2	77.2	1069
% CV	10.2	5.7	9.5	14.7	12.4	10.9

Recovery and Linearity - Samples spiked with high concentrations of Endostatin were evaluated for recovery. Samples were serially diluted to evaluate assay linearity.

Recovery		
Sample Type	Average % Recovery	Range %)
Cell culture supernates	112	49-137
Urine	93	86-143

Linearity									
						Platelet-poor			
		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	EDTA plasma	Heparin plasma	Urine	Human milk
1:2	Average % of Expected	91	100	97	104	106	111	98	102
	Range (%)	80-106	85-117	83-110	96-112	99-119	104-119	81-113	81-143
1:4	Average % of Expected	87	99	96	100	114	116	95	105
	Range (%)	76-104	71-122	75-112	74-126	95-125	110-122	74-120	78-139
1:8	Average % of Expected	85	102	99	107	121	124	102	107
	Range (%)	74-100	77-128	78-117	78-121	114-125	121-139	65-136	84-118

Specificity - This assay recognizes natural and recombinant human Endostatin. The assay was tested for cross-reactivity and interference with the following factors. Less than 0.5% cross-reactivity and interference was observed.

Recombinant human:			Recombinant mouse:		Recombinant rat:	Recombinant porcine:	Recombinant human multiplex partners:
Angiopoietin-2	FGF-17	IGF-II R	EG-VEGF	HGF	EGF	GM-CSF	Angiogenin
Angiopoietin-4	FGF-18	IGFBP-1	EGF	HGF R	FGF basic		Angiopoietin-1
Angiopoietin-like 3	FGF-19	IGFBP-2	EGF R	IGF-I	FGF-BP		FGF acidic
Angiopoietin-like 4	FGF-20	KGF/FGF-7	FGF-8b	IGF-II	β-NGF		FGF basic
CTGF	FGF-21	MSP	FGF-8c	IGFBP-1	PDGF-BB		PDGF-AA
EG-VEGF	FGF-22	MSP-β	FGF-15	KGF/FGF-7	$VEGF_{164}$		PDGF-BB
FGF-3	FGF R1α	M-CSF	FGF-21	M-CSF			PIGF
FGF-4	FGF R3	β-NGF	FGF-23	PDGF-CC			Thrombospondin-2
FGF-5	FGF R4	PD-ECGF	FGF R3	PIGF-2			VEGF
FGF-6	Flt-3	PDGF-CC	Flt-3	Thrombospondin-1			VEGF-D
FGF-8a	Flt-3 Ligand	PDGF-DD	Flt-3 Ligand	$VEGF-B_{167}$			
FGF-8e	G-CSF	VEGF-C	G-CSF	VEGF R2			
FGF-8f	G-CSF R	VEGF R1	GM-CSF	VEGF R3			
FGF-9	GM-CSF	VEGF R2					
FGF-10	HB-EGF	VEGF R3					
FGF-11	HRG-α	Thrombospondin-1					
FGF-12	IGF-I	Thrombospondin-4					
FGF-13	IGF-I R						
FGF-16	IGF-II						

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.

Luminex Performance Assays afford the user the benefit of multianalyte analysis of biomarkers in a complex sample. For each sample type, a single, multipurpose diluent is used to optimize recovery, linearity, and reproducibility. Such a multipurpose 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.