

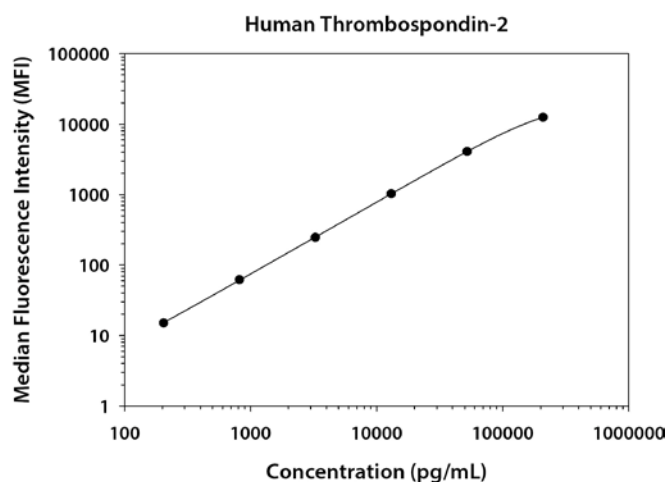
SPECIFICATIONS AND USE

- | | |
|---------------------------------|---|
| Recommended Sample Types | • Cell culture supernates, serum, EDTA plasma, heparin plasma, urine, and human milk. |
| Microparticle Region | • Region-21 |
| Components | <ul style="list-style-type: none"> • Microparticle Concentrate (Part 894460) is supplied as a 100X concentrated stock (0.075 mL) with preservatives. • Biotin-Antibody Concentrate (Part 893625) 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 | <ul style="list-style-type: none"> • 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 Thrombospondin-2 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, use the first six points for the Thrombospondin-2 kit (omit the lowest concentration standard).



Standard	pg/mL	MFI	Average	Corrected
Blank	0	10 10	10	—
1	209,000	12,468 12,499	12,484	12,474
2	52,250	4067 4111	4089	4079
3	13,063	1019 1049	1034	1024
4	3266	254 259	257	247
5	816	71 73	72	62
6	204	25 25	25	15

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 MFI of twenty zero standard replicates and calculating the corresponding concentration.

Thirty assays were evaluated, and the MDD of human Thrombospondin-2 ranged from 4.58-6.71 pg/mL. The mean MDD was 5.48 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)	416	1525	26,775		457	1667	26,557
Standard Deviation	35.7	69.5	1634		74.7	210	2234
% CV	8.6	4.6	6.1		16.3	12.6	8.4

Recovery and Linearity – Samples spiked with high concentrations of Thrombospondin-2 were evaluated for recovery. Samples were serially diluted to evaluate assay linearity.

Recovery			Linearity								
Sample Type	Average % Recovery	Range %			Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Platelet-poor		Urine
									EDTA plasma	Heparin plasma	
Cell culture supernates	102	84-135	1:2	Average % of Expected	94	100	97	103	103	108	97
Serum	105	78-141		Range (%)	90-100	88-108	91-108	93-115	97-109	104-111	93-101
EDTA plasma	101	70-125	1:4	Average % of Expected	88	88	92	96	113	114	92
Heparin plasma	103	73-167		Range (%)	79-94	73-96	76-110	79-112	98-120	109-119	78-99
PP EDTA plasma	75	65-91	1:8	Average % of Expected	87	93	86	89	114	117	85
PP Heparin plasma	80	40-97		Range (%)	76-97	66-127	60-103	76-102	108-123	108-123	68-100
Urine	99	90-103									

Specificity - This assay recognizes natural and recombinant human Thrombospondin-2. 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	EG-VEGF	EGF	GM-CSF	Angiogenin
Angiopoietin-4	EGF	FGF basic		Angiopoietin-1
Angiopoietin-like 3	EGF R	FGF-BP		Endostatin
Angiopoietin-like 4	FGF-8b	β -NGF		FGF acidic
CTGF	FGF-8c	PDGF-BB		FGF basic
EG-VEGF	FGF-15	VEGF ₁₆₄		PDGF-AA
FGF-3	FGF-21			PDGF-BB
FGF-4	FGF-23			PIGF
FGF-5	FGF R3			VEGF
FGF-6	Flt-3	Thrombospondin-1		VEGF-D
FGF-8a	Flt-3 Ligand	VEGF-B ₁₆₇		
FGF-8e	G-CSF	VEGF R2		
FGF-8f	G-CSF R	VEGF R3		
FGF-9	GM-CSF			
FGF-10	HB-EGF			
FGF-11	HRG- α			
FGF-12	IGF-I			
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.

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