Magnetic Luminex® Performance Assay Human CCL5/RANTES Kit

SPECIFICATIONS AND USE

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Recommended Sample Types	• Cell culture supernates, serum, EDTA plasma, and heparin plasma.
Microparticle Region	• Region-36
Components	• Microparticle Concentrate (Part 894448) is supplied as a 100X concentrated stock (0.075 mL) with preservatives.
	• Biotin-Antibody Concentrate (Part 892635) is supplied as a 100X concentrated stock solution (0.075 mL) with preservatives.
Other Supplies Required	• Magnetic Luminex Performance Assay Human Base Kit A (Catalog Number LUHM000).
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 Luminex Performance Assay procedure.

TYPICAL DATA

This human RANTES 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.
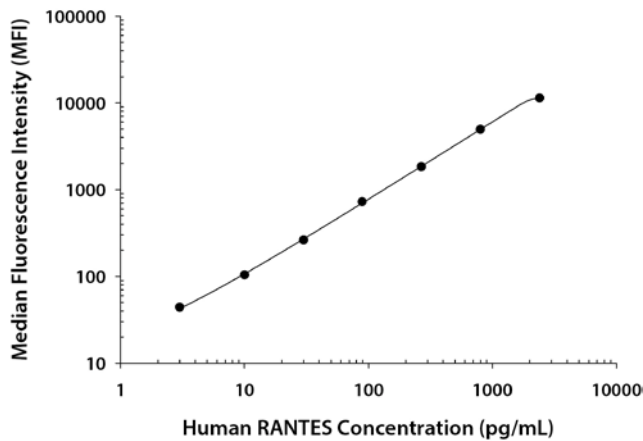
Standard	pg/mL	MFI	Average	Corrected
Blank	0	196 207	202	——
1	2400	11,495 11,638	11,567	11,365
2	800	5109 5186	5148	4946
3	267	2032 2049	2041	1839
4	89	918 933	926	724
5	30	461 466	464	262
6	10	301 311	306	104
7	3	242 249	246	44

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.

Eighteen assays were evaluated, and the MDD of human RANTES ranged from 0.59-1.91 pg/mL. The mean MDD was 1.08 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 twenty separate assays to assess precision between assays.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	198	492	988	212	473	938
Standard Deviation	13	49	121	17	57	123
% CV	6.4	10.0	12.2	7.9	12.1	13.1

Recovery and Linearity – Samples containing and/or spiked with high concentrations of RANTES were evaluated for recovery and were serially diluted to evaluate assay linearity.

Recovery		
Sample Type	Average % Recovery	Range (%)
Cell culture supernates	95	78-114

Linearity						
		Cell culture supernates	Serum	EDTA Plasma	Heparin Plasma
1:2	Average % of Expected	95	100	102	114
	Range (%)	80-104	98-102	90-126	102-130
1:4	Average % of Expected	96	104	97	124
	Range (%)	83-105	102-106	81-116	104-152
1:8	Average % of Expected	99	108	93	123
	Range (%)	86-111	107-108	84-104	106-152

Specificity - This assay recognizes natural and recombinant human RANTES. 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:	
6Ckine	IL-1 RII	IL-17	G-CSF	IL-8	GM-CSF	GM-CSF	ENA-78	IL-6
CNTF	IL-2 Rα	IL-18	GM-CSF	IL-10	IFN-γ	IL-1α	FGF basic	IL-8
β-ECGF	IL-2 Rβ	LIF	IFN-γ	IL-17	IL-1α	IL-1β	G-CSF	IL-10
FGF acidic	IL-2 Rγ	LIF R	IL-1α	MIP-1α	IL-1β	IL-2	GM-CSF	IL-17
FGF-4	IL-3 Rα	MIP-1α	IL-1ra	MIP-1β	IL-2	IL-4	IFN-γ	MCP-1
FGF-5	IL-4 R	MIP-3α	IL-1	RANTES	IL-4	IL-6	IL-1α	MIP-1α
FGF-6	IL-5 Rα	MIP-3β	IL-2	Tpo	IL-6	IL-8	IL-1β	MIP-1β
FGF-9	IL-6 R	MCP-2	IL-4	TNF-α	IL-10	IL-10	IL-1ra	Tpo
FGF-10	IL-10 R	MCP-3	IL-5	VEGF	TNF-α	Leptin	IL-2	TNF-α
FGF-18	IL-3	MCP-4	IL-6			TNF-α	IL-4	VEGF
GCP-2	IL-7	M-CSF					IL-5	
GROα	IL-9	TNF RI						
GROβ	IL-11	TNF-α						
GROγ	IL-12 p40	VEGF$_{121}$						
I-309	IL-12 p70	VEGF$_{165}$						
IGF-I	IL-13	VEGF-D						
IGF-II	IL-15							
IL-1 RI	IL-16							

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.