

Technical Data Certificate of Analysis

Caution: For Laboratory Use. A research chemical for research purposes only.

Membrane Target Systems™ human Chemokine CX₃CR1 Receptor

PRODUCT No.: ES-137-M000UA

LOT No.: 418-977-AL

MATERIAL PROVIDED

MEMBRANES:	1 x 80000 units / 10 ml frozen aliquot.
PACKAGING BUFFER:	50 mM Tris-HCl (pH 7.4), 10% sucrose.
STORAGE CONDITIONS:	Store at -80°C. Avoid repeated freeze-thaw.

PRODUCT INFORMATION

CELLULAR BACKGROUND:	CHO-K1
PROTEIN ACCESSION NUMBER:	U20350
UNIT SIZE:	0.5 µg protein / unit
STABILITY:	This product is stable for at least 3 years from reception if used and stored under recommended conditions.

QUALITY CONTROL

EXPRESSION LEVEL (B_{Max}):	0.22 pmol/mg membrane protein.
K_D FOR [¹²⁵I]-FRACTALKINE :	0.0022 nM
PROTEIN CONCENTRATION:	4 mg/ml

RECOMMENDED ASSAY CONDITIONS

ASSAY BUFFER:	25 mM Hepes pH 7.4, 10 mM MgCl ₂ , 1 mM CaCl ₂ , 0.5% BSA
WASH BUFFER:	25 mM Hepes pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 500 mM NaCl
BINDING PROTOCOL:	Binding assays are performed in 200 µl total volume according to the following conditions: 1 - Membrane dilution: 6.25 µl of membranes + 7.49 ml assay buffer (1:1200 dilution) 2 - Incubation: 25 µl of incubation buffer or unlabeled ligand (Fractalkine/CX ₃ CL1 (human) , 0.1 µM final for non specific binding) 25 µl of radioligand at the appropriate concentration (see graph on page 2) 150 µl of diluted membranes 3 - Incubation time: 60 minutes at 27 °C 4 - Filtration: Over GF/B filter presoaked in 0.5 % PEI then washed 9x with 500 µl of ice cold wash buffer.

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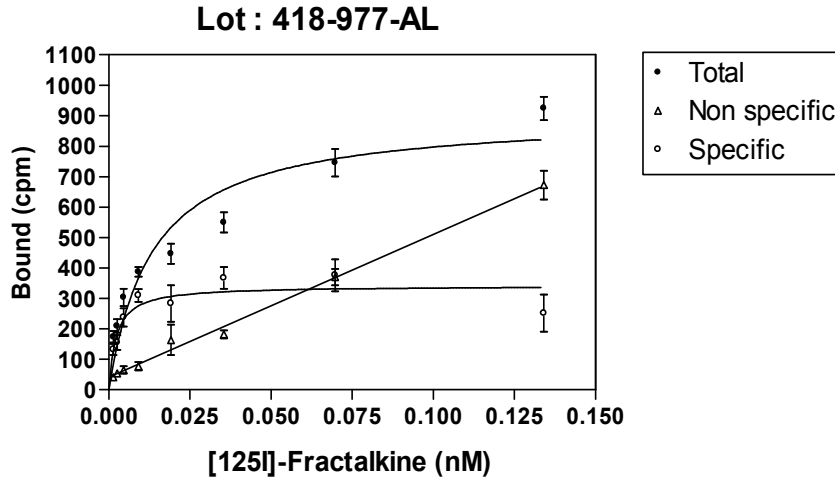


Figure 1: SATURATION BINDING ASSAY CURVE (FILTRATION)
 96-well saturation binding assay curve (0.5 µg membranes/well, TopCount™).

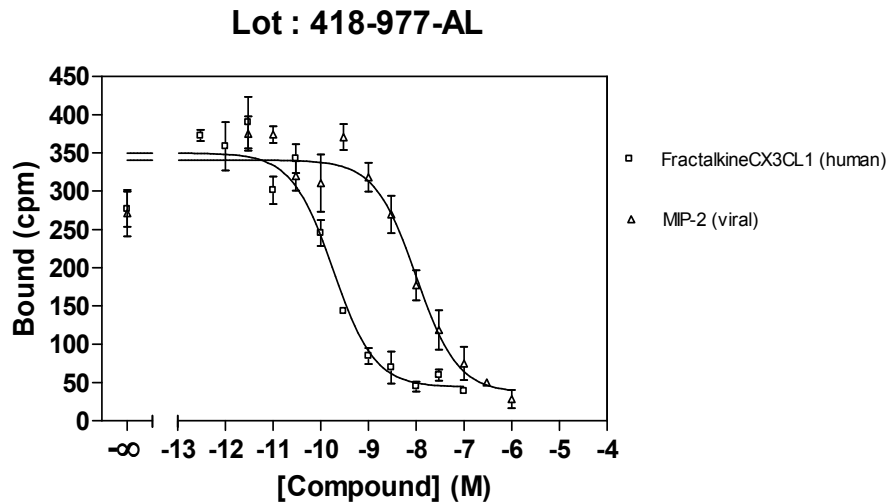
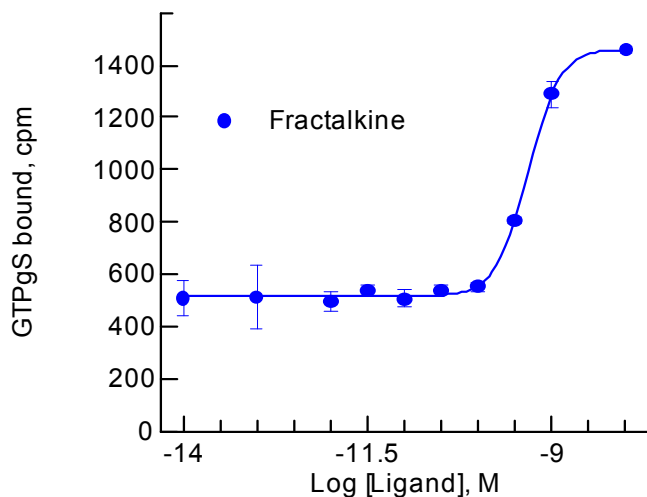


Figure 2: COMPETITION BINDING ASSAY CURVE (FILTRATION)
 96-well competition binding assay curve (0.5 µg membranes/well, TopCount™). Recommended radioligand concentration = 0.01 nM.

*Even though two sites can be observed occasionally with some ligands, the data presented is derived from single site fitting.

Reference Compounds	Ki (nM)
Fractalkine/CX3CL1 (human)	0.03
MIP-2 (viral)	1.8

PRODUCT TYPICAL DATA



Agonist / Antagonist	EC50 / IC ₅₀ (M)
Fractalkine	4.8 x 10 ⁻¹⁰

Figure 3: GTPγS³⁵ FUNCTIONAL ASSAY

GTPγS³⁵ assays were performed using homogeneous proximity assay method.

SUGGESTED MATERIALS AND INSTRUMENTATION

PHARMACOLOGY ASSAYS

- [¹²⁵I]-Fractalkine
- Fractalkine/CX3CL1 (human)
- Unifilter-96 GF/C
- Unifilter-96 GF/B
- Filtermat A GF/C

SUPPLIER

Amersham
Peprotech
PerkinElmer
PerkinElmer
PerkinElmer

CATALOG

IM326
300-31
6005174
6005177
1450-421

INSTRUMENTS

- TopCount™
- Microbeta®
- FilterMate Unifiter 96-Harvester

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