

Technical Data Certificate of Analysis

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

Membrane Target Systems™ human Opioid mu Receptor

PRODUCT No.: ES-542-M400UA
LOT No.: 526-408-A

MATERIAL PROVIDED

MEMBRANES: 1 x 400 units / 400 µl frozen aliquot.
PACKAGING BUFFER: 50 mM Tris-HCl (pH 7.4), 0.5 mM EDTA, 10 mM MgCl₂, 10% sucrose.

PRODUCT INFORMATION

CELLULAR BACKGROUND: CHO-K1
PROTEIN ACCESSION NUMBER: L29301
UNIT SIZE: 5 µg protein / unit
STORAGE CONDITIONS: Store at -80°C. **Freeze-thaw is not recommended** as it can affect product performance and homogeneity. In order to minimize negative impact of freeze-thawing, flash freeze in liquid nitrogen for 30 seconds prior to transferring to -80°C.
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}): 1.8 pmol/mg membrane protein.
K_D FOR [³H]-DAMGO : 0.23 nM
PROTEIN CONCENTRATION: 5 mg/ml

RECOMMENDED ASSAY CONDITIONS

ASSAY BUFFER: 50 mM Tris-HCl pH 7.4, 5 mM MgCl₂
WASH BUFFER: 50 mM Tris-HCl pH 7.4
BINDING PROTOCOL: Binding assays are performed in 550 µl total volume according to the following conditions:
1 - Membrane dilution: 0.05 ml of membranes + 24.95 ml assay buffer (1:500 dilution)
2 - Incubation: 25 µl of incubation buffer or unlabeled ligand (DAMGO, 50 µM final for non specific binding)
25 µl of radioligand at the appropriate concentration (see graph on page 2)
500 µl of diluted membranes
3 - Incubation time: 60 minutes at 27 °C
4 - Filtration: Over GF/B filter presoaked in 0.5 % BSA then washed 9x with 500 µl of ice cold wash buffer.

LOT SPECIFIC DATA

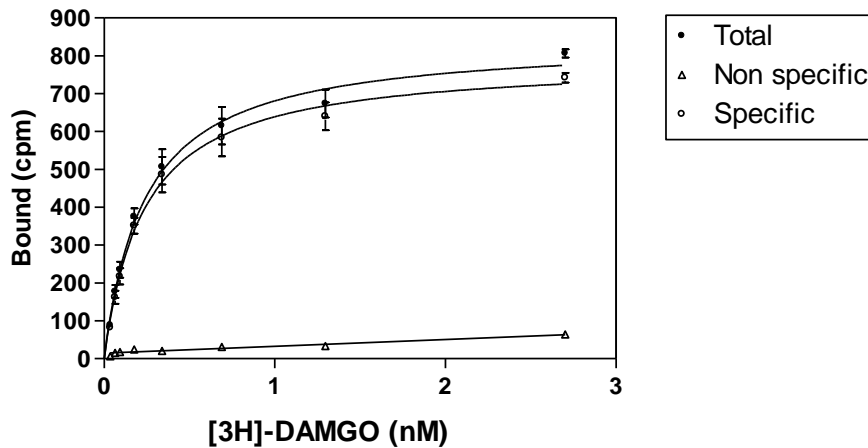


Figure 1: SATURATION BINDING ASSAY CURVE (FILTRATION)
96-well saturation binding assay curve (5 μ g membranes/well, TopCountTM).

TYPICAL PRODUCT DATA

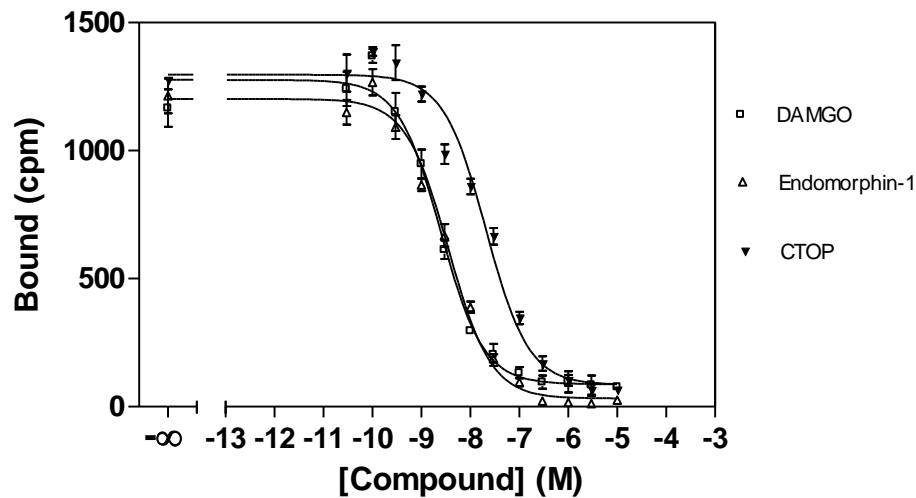
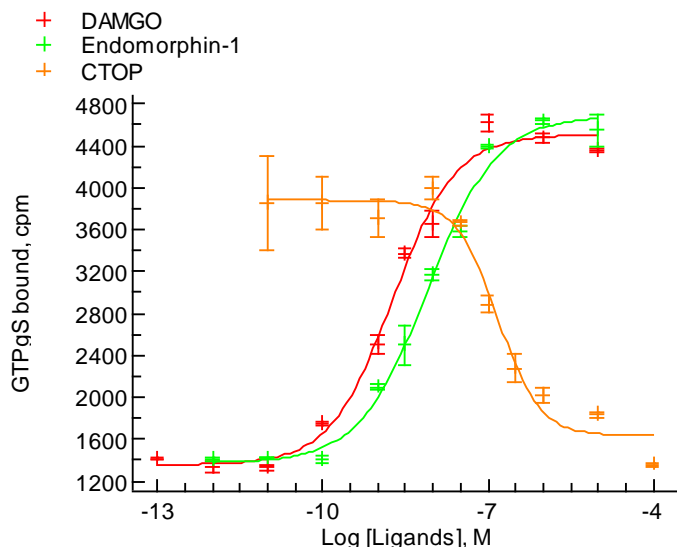


Figure 2: COMPETITION BINDING ASSAY CURVE (FILTRATION)
96-well competition binding assay curve (5 μ g membranes/well, TopCountTM). Recommended radioligand concentration = 0.75 nM.

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

Reference Compounds	K _i (nM)
DAMGO	1.0
Endomorphin-1	1.5
CTOP	8.7



Agonist / Antagonist	EC ₅₀ / IC ₅₀ (nM)
DAMGO	1.8
Endomorphin-1	7.9
CTOP	150

Figure 3: GTPγS³⁵ FUNCTIONAL ASSAY

GTPγS³⁵ assays were performed using homogeneous proximity assay method.

SUGGESTED MATERIALS AND INSTRUMENTATION

	SUPPLIER	CATALOG #
PHARMACOLOGY ASSAYS		
• [³ H]-DAMGO	PerkinElmer	NET902
• DAMGO	Sigma	E7384
• Unifilter-96 GF/C	PerkinElmer	6005174
• Unifilter-96 GF/B	PerkinElmer	6005177
• Filtermat A GF/C	PerkinElmer	1450-421
INSTRUMENTS		
• TopCount™	PerkinElmer	visit the web site or contact your local sales office
• Microbeta®	PerkinElmer	visit the web site or contact your local sales office
• FilterMate Unifiter 96-Harvester	PerkinElmer	C961962

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