

# 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.: 479-871-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 MgCl<sub>2</sub>, 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<sub>Max</sub>):** 2.7 pmol/mg membrane protein.

**K<sub>D</sub> FOR [<sup>3</sup>H]-DAMGO :** 0.3 nM

**PROTEIN CONCENTRATION:** 5 mg/ml

#### RECOMMENDED ASSAY CONDITIONS

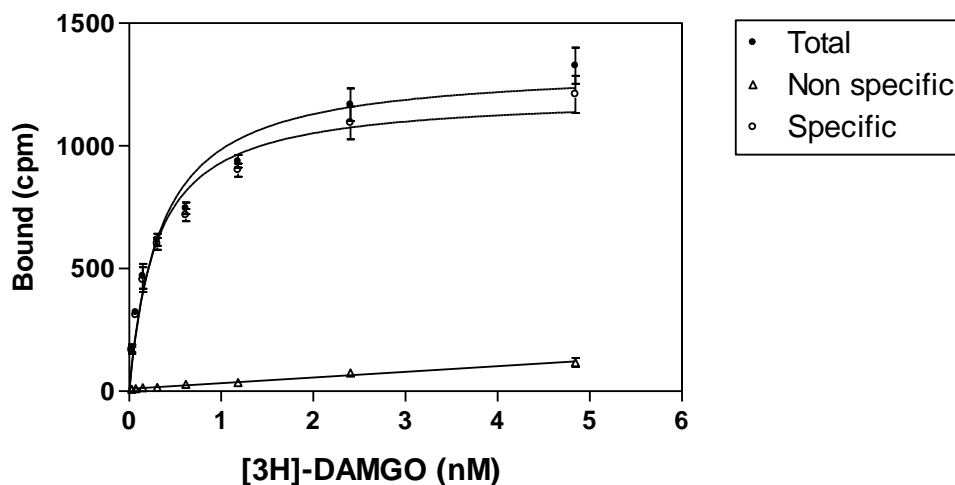
**ASSAY BUFFER:** 50 mM Tris-HCl pH 7.4, 5 mM MgCl<sub>2</sub>

**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:

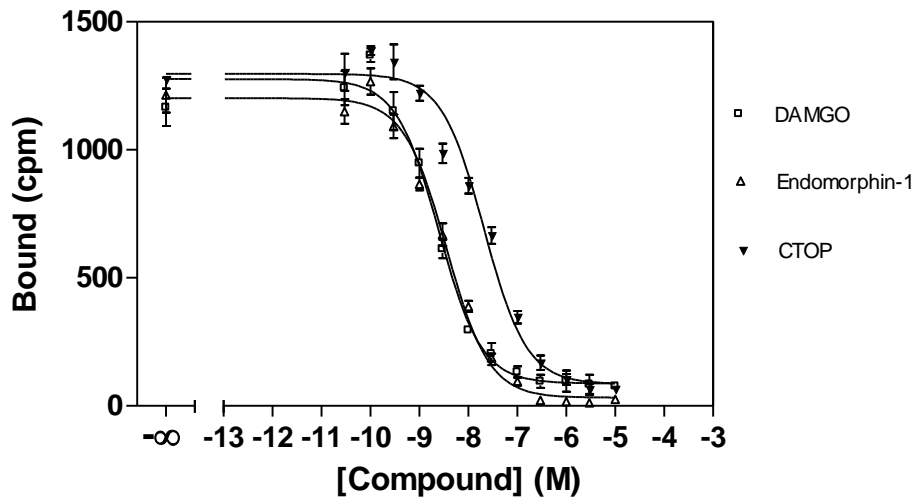
- 1 - Membrane dilution: 0.05 ml of membranes + 24.95 ml assay buffer (1:500 dilution)
- 2 - Incubation: 25 µl of incubation buffer or unlabeled ligand (DAMGO, 50 µM final for non specific binding)  
25 µl of radioligand at the appropriate concentration (see graph on page 2)  
500 µl of diluted membranes
- 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  $\mu$ g membranes/well, TopCount™).

## TYPICAL PRODUCT DATA



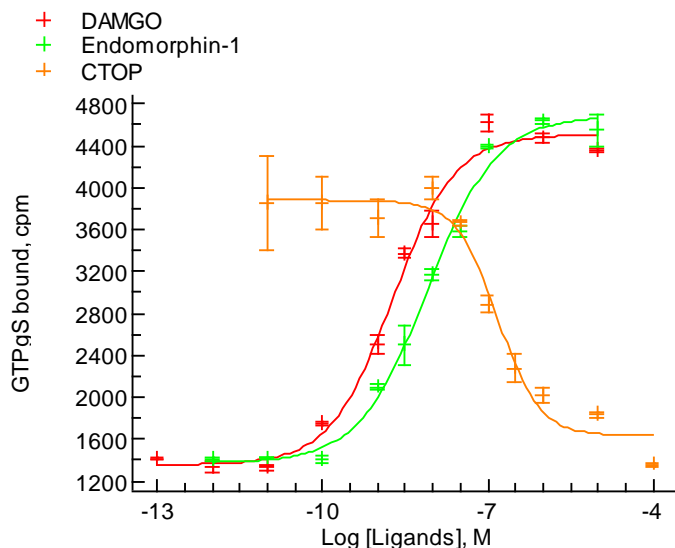
**Figure 2: COMPETITION BINDING ASSAY CURVE (FILTRATION)**  
96-well competition binding assay curve (5  $\mu$ g membranes/well, TopCount™). Recommended radioligand concentration = 0.75 nM.

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

Reference Compounds	K <sub>i</sub> (nM)
DAMGO	1.0
Endomorphin-1	1.5



CTOP	8.7



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

**Figure 3: GTPγS<sup>35</sup> FUNCTIONAL ASSAY**

GTPγS<sup>35</sup> assays were performed using homogeneous proximity assay method.

## SUGGESTED MATERIALS AND INSTRUMENTATION

	SUPPLIER	CATALOG #
<b>PHARMACOLOGY ASSAYS</b>		
• [ <sup>3</sup> 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
<b>INSTRUMENTS</b>		
• 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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