

Research use only. Not for use in diagnostic procedures.

Membrane Target Systems™

# human Opioid mu Receptor

Product No.: ES-542-M400UA

Lot No.: 2916194

#### Material Provided

Membranes:  $1 \times 400 \text{ units} / 400 \mu \text{L}$  frozen aliquot

#### **Product Information**

Cellular Background: CHO-K1

GenBank Accession Number: L29301

Unit Size: 5 µg protein / unit

Storage Buffer: 50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM MgCl<sub>2</sub>, 10% sucrose.

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**

 $B_{\text{max}}$  and  $K_{\text{d}}$  are determined using radioactive saturation binding assays (Figure 1). Protein concentration is determined using the BCA method <sup>(1)</sup>. Ratio-to-Reference (RTR) is determined by dividing the maximal signal of the current lot ( $B_{\text{max}}$  in fmoles) by the maximal signal of a pre-defined reference tested in parallel. RTR is an indicator of lot-to-lot consistency. \*We certify that these results meet our quality release criteria.

Ratio-to-Reference (RTR): 0.77

Expression Level (B<sub>max</sub>): 6.8 pmol/mg membrane protein.

 $K_d$  for [ $^3$ H]-DAMGO: 0.14 nM

Protein Concentration: 5 µg/µL

(1) Smith, P.K., et al. (1985). Anal. Biochem. 150, 76-85.



### **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 DAMGO (Sigma E7384) 50 μM final for non

specific binding (Saturation binding assay)

For competition binding assay: 25  $\mu L$  of reference compounds at

decreasing concentrations (see figure 2)

25 µL of radioligand at the appropriate concentration (see graph below)

500 µL of diluted membranes

3 - Incubation time: 60 minutes at 27 °C

4 - Filtration: aspirate and wash 9 x 500  $\mu$ L with ice cold wash buffer over GF/C filter

(presoaked in 0.5 % BSA).

## Lot Specific Data

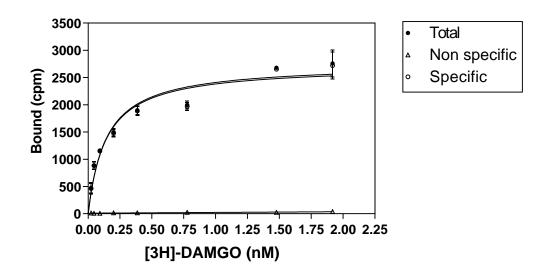


Figure 1: Saturation binding assay curve (filtration) 96-well saturation binding assay curve (5 µg membranes/well, TopCount®) using [³H]-DAMGO (Revvity NET902 Lot No.: 2916194)



## Typical Product Data

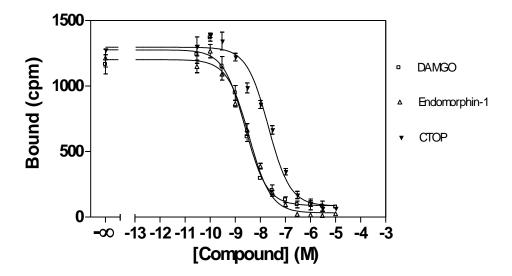


Figure 2: Competition binding assay curve (filtration) 96-well competition binding assay curve (5  $\mu$ g membranes/well, TopCount®). Recommended radioligand concentration = 0.75 nM.

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

Reference Compounds	Ki
	(nM)
DAMGO	1.0
Endomorphin-1	1.5
СТОР	8.7

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