Membrane Target Systems™

human Galanin Gal$_2$ Receptor

Product No.:   ES-511-M400UA

Lot No.:   2121985

Material Provided

Membranes:	1 x 400 units / 400 µL frozen aliquot

Product Information

Cellular Background:	CHO-K1
GenBank Accession Number:	AF080586
Unit Size:	3 µg protein / unit
Storage Buffer:	50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM MgCl$_2$, 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$_{max}$ and K$_d$ are determined using radioactive saturation binding assays (Figure 1). Protein concentration is determined using the BCA method [1]. Ratio-to-Reference (RTR) is determined by dividing the maximal signal of the current lot (B$_{max}$ 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):	N/A
Expression Level (B$_{max}$):	1.07 pmol/mg membrane protein.
K$_d$ for [^{125}I]-Galanin (human):	2.06 nM
Protein Concentration:	3 µg/µL

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

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Recommended Assay Conditions

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Assay Buffer:	25 mM Hepes pH 7.4, 5 mM MgCl$_2$, 1 mM EDTA, 0.5% BSA
Wash Buffer:	25 mM Hepes pH 7.4, 5 mM MgCl$_2$, 1 mM EDTA, 0.5% BSA
Binding Protocol:	Binding assays are performed in 200 µL total volume according to the following conditions:
1 - Membrane dilution:	0.05 mL of membranes + 7.45 mL assay buffer (1: 150 dilution)
2 - Incubation:	25 µL of incubation buffer or Galanin (human) (Bachem H-8230) 5 µM final for non specific binding (Saturation binding assay)
	For competition binding assay: 25 µL of reference compounds at decreasing concentrations (see figure 2)
	25 µL of radioligand (diluted 1:10 isotopically for saturation experiments) at the appropriate concentration (see graph below) 150 µL of diluted membranes
3 - Incubation time:	60 minutes at 27 °C
4 - Filtration:	aspirate and wash 9 x 500 µL with ice cold wash buffer over GF/C filter (presoaked in 0.5 % PEI).

Lot Specific Data
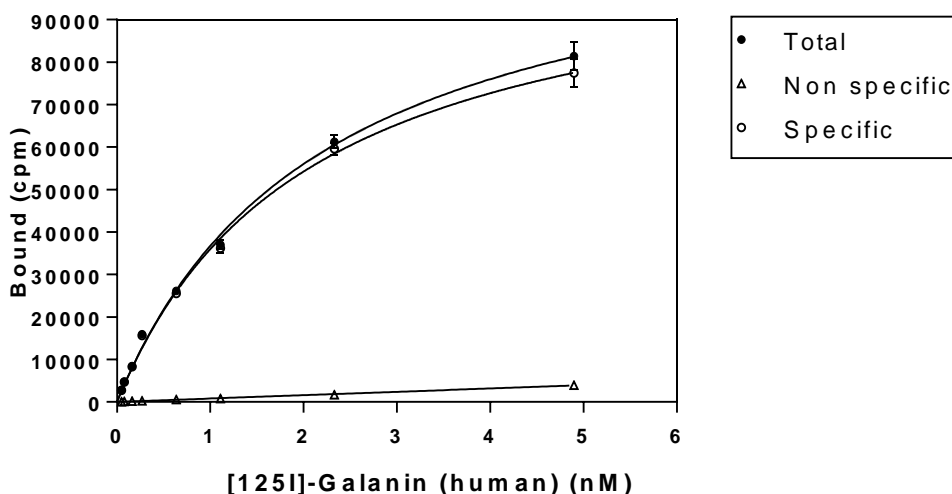
Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (3 µg membranes/well, TopCount®) using [^{125}I]-Galanin (human) (Revvity NEX333 Lot No.: HP32560)
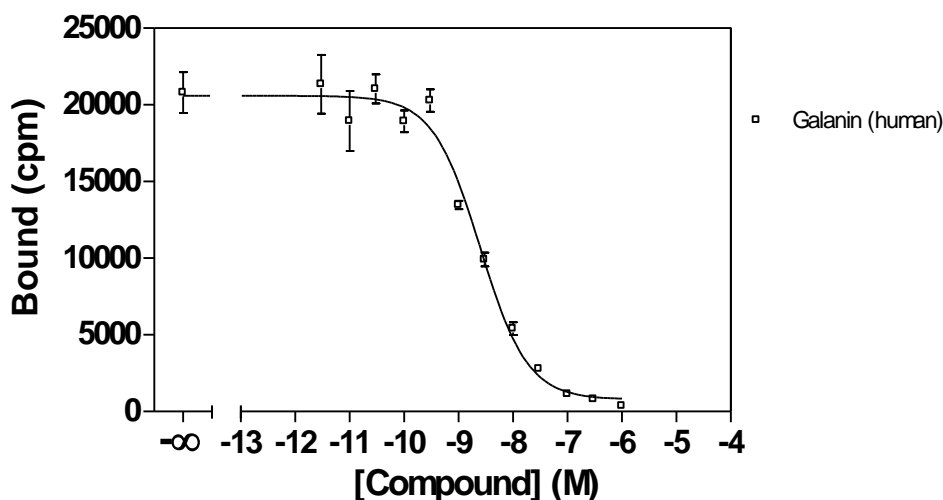
Figure 2: Competition binding assay curve (filtration)
96-well competition binding assay curve (3 µg membranes/well, TopCount®). Recommended radioligand concentration = 1.0 nM.

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

Reference Compounds	Ki (nM)
Galanin (human)	1.57

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