human Glutamate $mGlu_{5A}$ Receptor

Product No.: ES-555-M400UA

Lot No.: 3109516

Material Provided

Membranes:	1 x 400 units / 1000 µL frozen aliquot

Product Information

Cellular Background:	CHO-K1
GenBank Accession Number:	D28538
Unit Size:	15 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):	0.85
Expression Level (B_{max}):	1.65 pmol/mg membrane protein.
K_d for [^{3}H]-Quisqualic acid:	14 nM
Protein Concentration:	6 µg/µL

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

Recommended Assay Conditions

Assay Buffer:	25 mM Hepes pH 7.4, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$
Wash Buffer:	25 mM Hepes pH 7.4, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$
Binding Protocol:	Binding assays are performed in 550 µL total volume according to the following conditions:
1 - Membrane dilution:	0.125 mL of membranes + 24.875 mL assay buffer (1:200 dilution)
2 - Incubation:	25 µL of incubation buffer or L-Quisqualic acid (Tocris 0188) 200 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 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 µL with ice cold wash buffer over GF/C filter (presoaked in 25 mM Hepes pH 7.4, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$).

Lot Specific Data

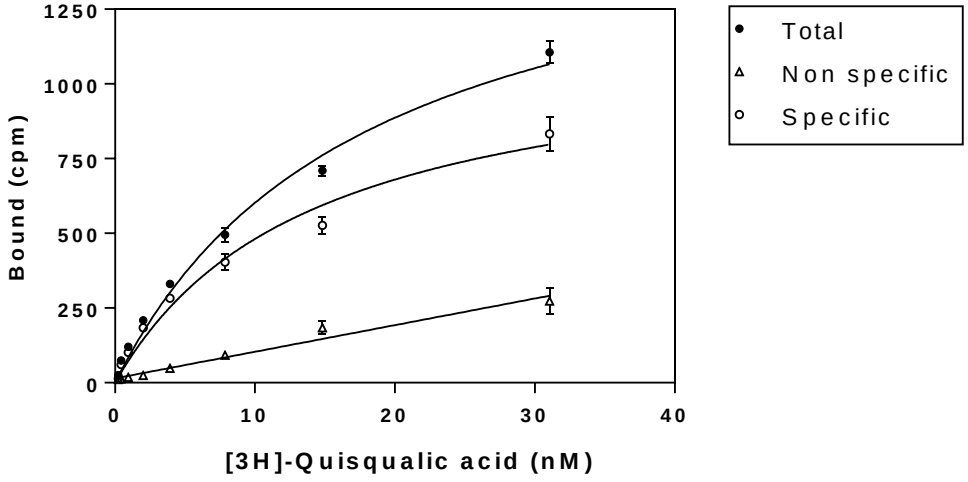

Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (15 µg membranes/well, TopCount®) using [^{3}H]-Quisqualic acid (Revvity NET1165 Lot No.: 3007672)

Typical Product Data

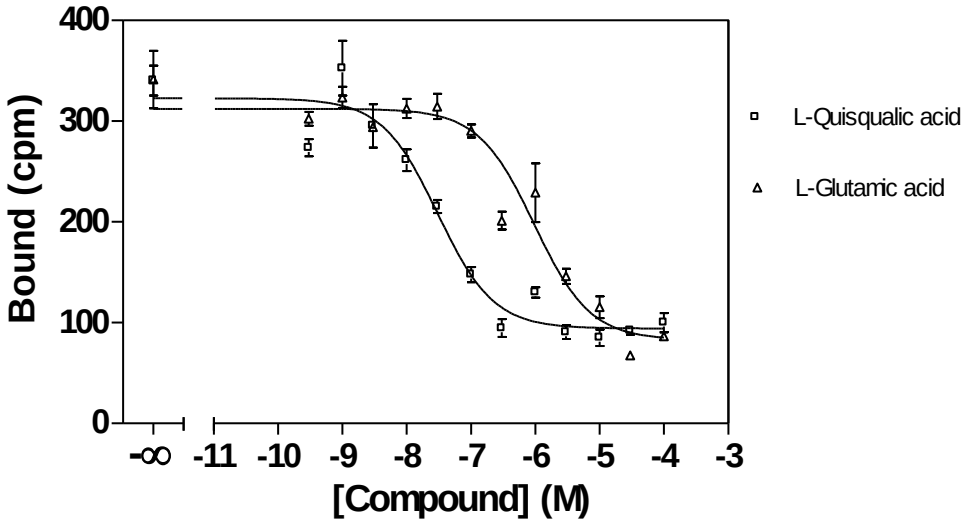

Figure 2: Competition binding assay curve (filtration)
96-well competition binding assay curve (15 µg membranes/well, TopCount®). Recommended radioligand concentration = 40 nM.

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

Reference Compounds	Ki (nM)
L-Quisqualic acid	22
L-Glutamic acid	708

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