

## human Adenosine A<sub>3</sub> Receptor

Product Number: ES-012-M400UA

Lot Number: 2787879

### Material Provided

Membranes: 1 x 400 units / 400 µL frozen aliquot

### Product Information

Cellular Background: CHO-K1

GenBank Accession Number: L22607

Unit Size: 1.5 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<sub>max</sub> and K<sub>d</sub> 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<sub>max</sub> 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<sub>MAX</sub>): 2.2 pmol/mg membrane protein.

K<sub>D</sub> for [<sup>125</sup>I]-AB-MECA: 0.86 nM

Protein Concentration: 1.5 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, 10 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.5% BSA

Wash Buffer: 50 mM Tris-HCl pH 7.4

Binding Protocol: Binding assays are performed in 200  $\mu$ L 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  $\mu$ L of incubation buffer or IB-MECA (Sigma I146) 250  $\mu$ M 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  $\mu$ L of radioligand at the appropriate concentration (see graph below)  
150  $\mu$ L 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

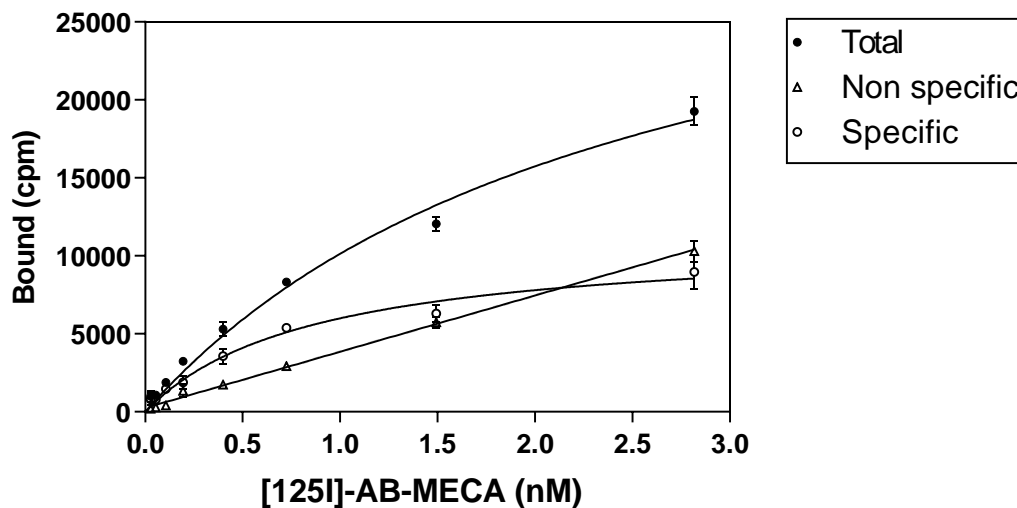


Figure 1: Saturation binding assay curve (filtration)  
96-well saturation binding assay curve (1.5  $\mu$ g membranes/well, TopCount®) using [<sup>125</sup>I]-AB-MECA (Revvity NEX312 Lot No.: GU41700)

## Typical Product Data

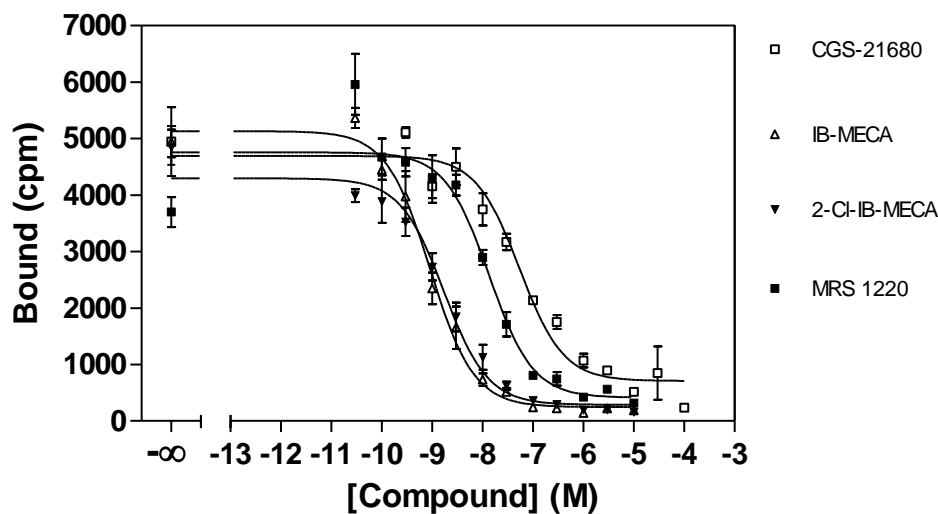


Figure 2: Competition binding assay curve (filtration)  
96-well competition binding assay curve (1.5  $\mu$ g membranes/well, TopCount®). Recommended radioligand concentration = 0.4 nM.

\*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)
CGS-21680	43
IB-MECA	0.73
2-Cl-IB-MECA	1.4
MRS 1220	11

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