

human Adenosine A_{2B} Receptor

Product Number: **ES-013-M400UA**

Lot Number: 2988775

Material Provided

Membranes: 1 x 400 units / 1000 µL frozen aliquot

Product Information

Cellular Background: HEK293

GenBank Accession Number: M97759

Unit Size: 20 µg protein / unit

Storage Buffer: 50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM MgCl₂, 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): 0.73

Expression Level (B_{MAX}): 0.55 pmol/mg membrane protein.

K_D for [³H]-Cyclopentyl-1,3-dipropylxanthine: 8.04 nM

Protein Concentration: 8 µg/µL

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

Recommended Assay Conditions

Assay Buffer: 50 mM Hepes pH 7.4, 5 mM MgCl₂, 1 mM EDTA

Wash Buffer: 5 mM Hepes pH 6.5, 5 mM MgCl₂, 1 mM EDTA, 0.25% BSA

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 5'-(N-Ethylcarboxamido)adenosine (Sigma E2387) 100 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 0.5 % BSA).

Lot Specific Data

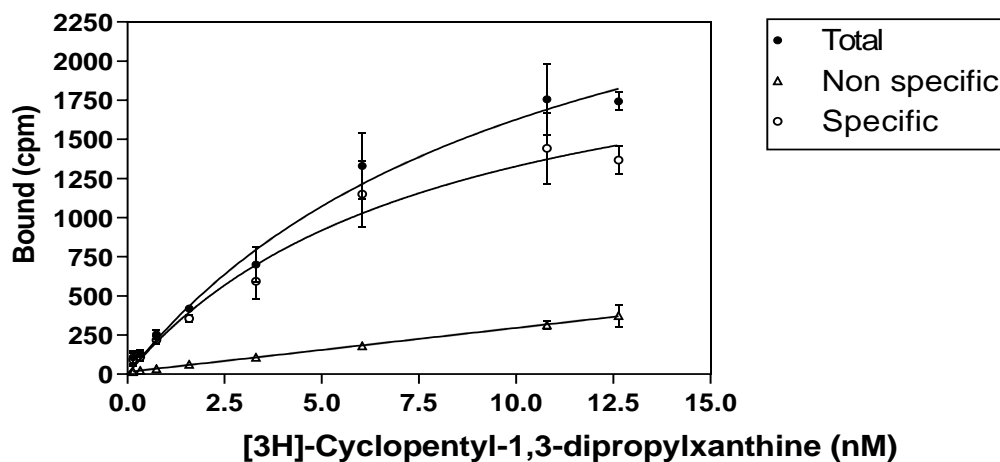


Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (20 μg membranes/well, TopCount®) using [³H]-Cyclopentyl-1,3-dipropylxanthine (Revvity NET974 Lot No.: 2926483)

Typical Product Data

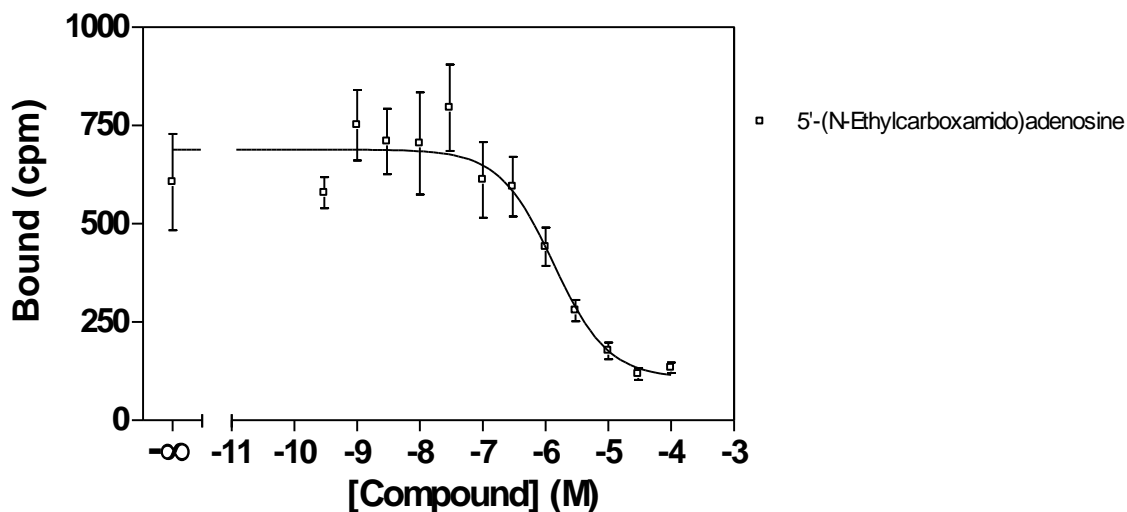


Figure 2: Competition binding assay curve (filtration)
96-well competition binding assay curve (20 μ g membranes/well, TopCount®). Recommended radioligand concentration = 5 nM.

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

Reference Compounds	K _i (nM)
5'-(N-Ethylcarboxamido)adenosine	880

This product is not for resale or distribution except by authorized distributors. The information provided in this document is valid for the specified lot number and date of analysis. This information is for reference purposes only and does not constitute a warranty or guarantee of the product's suitability for any specific use. Revvity, Inc., its subsidiaries, and/or affiliates (collectively, "Revvity") do not assume any liability for any errors or damages arising from the use of this document or the product described herein. REVVITY EXPRESSLY DISCLAIMS ALL WARRANTIES, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, REGARDLESS OF WHETHER ORAL OR WRITTEN, EXPRESS OR IMPLIED, ALLEGEDLY ARISING FROM ANY USAGE OF ANY TRADE OR ANY COURSE OF DEALING, IN CONNECTION WITH THE USE OF INFORMATION CONTAINED HEREIN OR THE PRODUCT ITSELF.