

## human Adrenergic $\alpha_{1A}$ Receptor

Product Number: ES-036-M400UA

Lot Number: 2226549

### Material Provided

Membranes: 1 x 400 units / 400  $\mu$ L frozen aliquot

### Product Information

Cellular Background: CHO-K1

GenBank Accession Number: U03866

Unit Size: 0.13  $\mu$ g 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>): 32.2 pmol/mg membrane protein.

K<sub>D</sub> for [<sup>125</sup>I]-HEAT: 0.5 nM

Protein Concentration: 0.13  $\mu$ g/ $\mu$ L

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

Recommended Assay Conditions

Assay Buffer: 25 mM HEPES, 10 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.5 % BSA; pH 7.4

Wash Buffer: 25 mM HEPES, 5 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.5 M NaCl; pH 7.4

Binding Protocol: Binding assays are performed in 200  $\mu$ L total volume according to the following conditions:

1 - Membrane dilution: 0.07 mL of membranes + 10.43 mL assay buffer (1:150 dilution)

2 - Incubation: 25  $\mu$ L of assay buffer or HEAT hydrochloride (Tocris 0535) 100  $\mu$ 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: 30 minutes at 37 °C

4 - Filtration: aspirate and wash 9 x 500  $\mu$ L with ice cold wash buffer over GF/C filter (presoaked in assay buffer).

Lot Specific Data

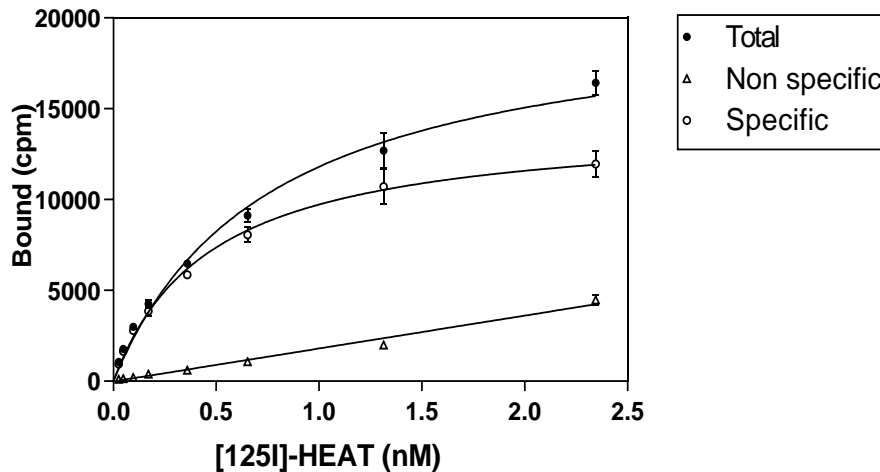


Figure 1: Saturation binding assay curve (filtration)  
96-well saturation binding assay curve (0.13  $\mu$ g membranes/well, TopCount®) using [<sup>125</sup>I]-HEAT (Revvity NEX182 Lot No.: CQ12260)

## Typical Product Data

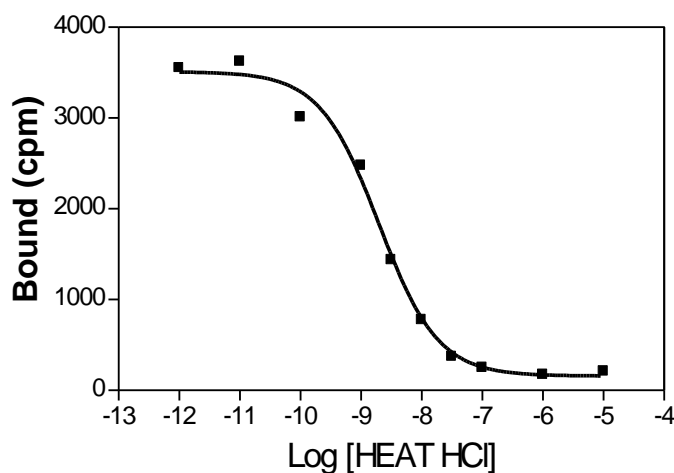


Figure 2: Competition binding assay curve (filtration)  
96-well competition binding assay curve (0.13  $\mu$ g membranes/well, TopCount<sup>®</sup>). Recommended radioligand concentration = 0.35 nM.

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

Reference Compounds	Ki (nM)
HEAT HCl	1.69

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