

human Angiotensin AT₂ Receptor

Product Number: ES-070-M400UA

Lot Number: 2204886

Material Provided

Membranes: 1 x 400 units / 400 µL frozen aliquot

Product Information

Cellular Background: CHO-K1

GenBank Accession Number: X70811

Unit Size: 2 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): 0.84

Expression Level (B_{MAX}): 0.41 pmol/mg membrane protein.

K_D for [¹²⁵I]-(-)Iodocyanopindolol: 0.01 nM

Protein Concentration: 2 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, 1 mM EDTA, 0.5% BSA

Wash Buffer: 10 mM Hepes pH 7.4, 500 mM NaCl

Binding Protocol: Binding assays are performed in 200 μ 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 μ L of incubation buffer or Cyanopindolol (Tocris 0993) 250 μ M final for non-specific binding (Saturation binding assay)

For competition binding assay: 25 μ L of reference compounds at decreasing concentrations (see figure 2)

25 μ L of radioligand at the appropriate concentration (see graph below)
150 μ L of diluted membranes

3 - Incubation time: 30 minutes at 27 $^{\circ}$ C

4 - Filtration: aspirate and wash 9 x 500 μ L with ice cold wash buffer over GF/B filter (presoaked in 0.5 % BSA).

Lot Specific Data

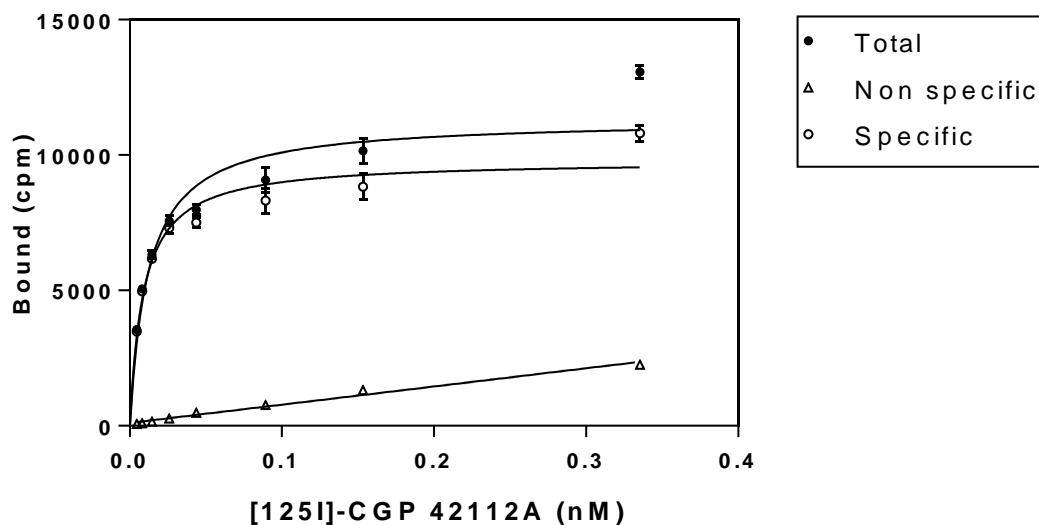


Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (2 μ g membranes/well, TopCount[®]) using [¹²⁵I]-(-)iodocyanopindolol (Revvity NEX189 Lot No.: HGB2360)

Typical Product Data

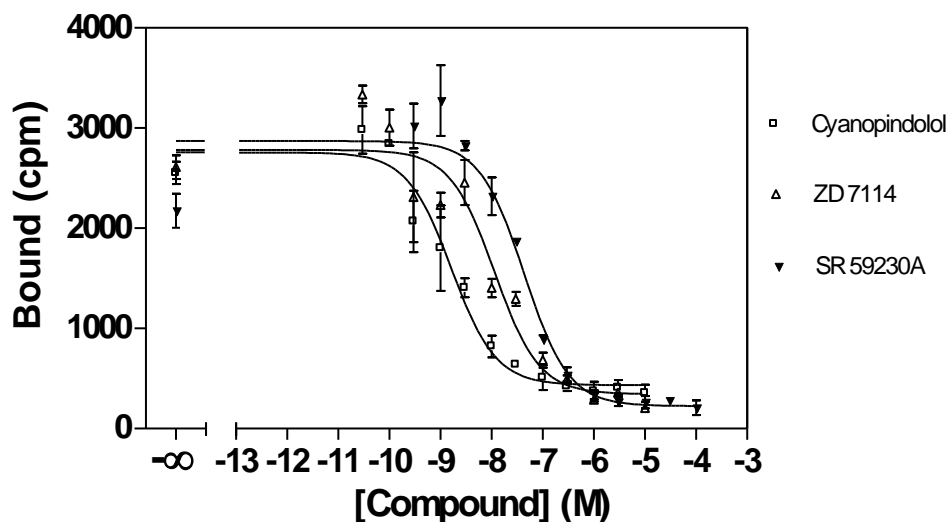


Figure 2: Competition binding assay curve (filtration)
96-well competition binding assay curve (2 μ g membranes/well, TopCount®). Recommended radioligand concentration = 0.165 nM.

*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)
Cyanopindolol	1.2
ZD 7114	8.6
SR 59230A	33

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