TECHNICAL DATA SHEET

Membrane Target Systems™

Caution: For Laboratory Use. A research reagent for research purposes only

human Angiotensin AT_1 Receptor

Product No.: ES-072-M400UA

Lot No.: 1772291

Material Provided

Membranes:	1 x 400 units / 400 µL frozen aliquot

Product Information

Cellular Background:	CHO-K1
GenBank Accession Number:	M91464
Unit Size:	0.6 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

Bmax and Kd 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 (Bmax 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_{MAX}):	3.3 pmol/mg membrane protein.
K_D for [^{125}I]-(Sar^1,Ile^8)-Angiotensin II :	0.11 nM
Protein Concentration:	0.6 µg/µL

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

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Recommended Assay Conditions

Assay Buffer: 50 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$

Wash Buffer: 50 mM Tris-HCl pH 7.4

Binding Protocol: Binding assays are performed in 200 µL 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 µL of incubation buffer or (Sar^1,Ile^8)-Angiotensin II (Bachem H-1730) 10 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) 150 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

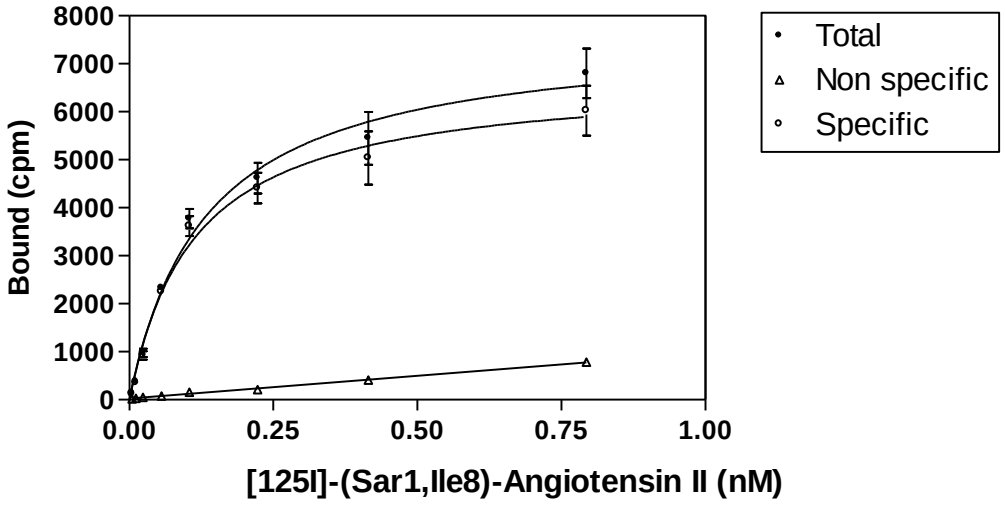

Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (0.6 µg membranes/well, TopCount®) using [^{125}I]-(Sar^1,Ile^8)-Angiotensin II (PerkinElmer NEX248 Lot No.: EJB1280)

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Typical Product Data

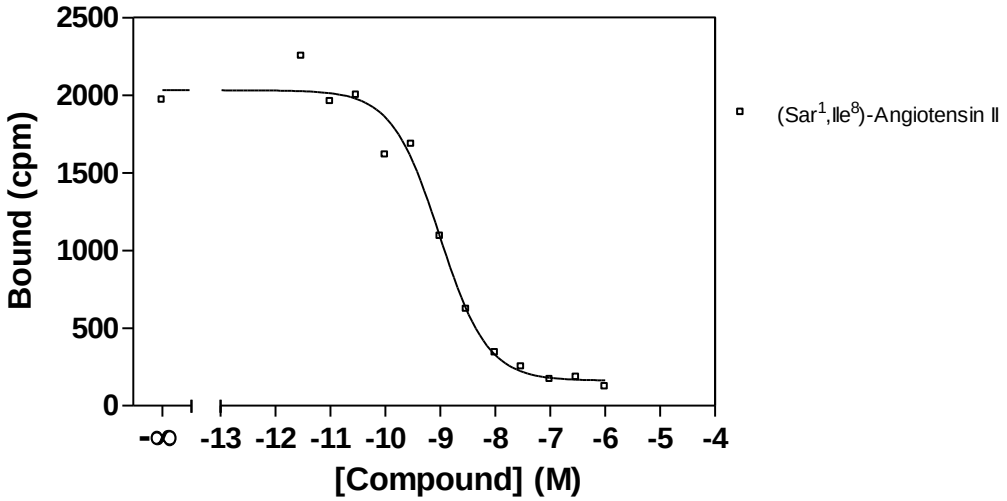

Figure 2: Competition binding assay curve (filtration)
96-well competition binding assay curve (0.6 µg membranes/well, TopCount®). Recommended radioligand concentration = 0.03 nM.

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

Reference Compounds	Ki (nM)
(Sar[1],Ile[8])-Angiotensin II	0.84

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Suggested Materials and Instrumentation

Please visit our website

www.perkinelmer.com/GPCR

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PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs