

## human Orexin OX<sub>1</sub> Receptor

Product No.: ES-330-M400UA

Lot No.: 2075250

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### Material Provided

Membranes: 1 x 400 units / 400 µL frozen aliquot

### Product Information

Cellular Background: CHO-K1

GenBank Accession Number: AF041243 (G280A)

Unit Size: 1 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

IC<sub>50</sub> is determined using a radioactive competition binding assay (Figure 1). Protein concentration is determined using the BCA method <sup>(1)</sup>. \*We certify that these results meet our quality release criteria.

IC<sub>50</sub> for Orexin A : 3.4 nM

Protein Concentration: 1 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, 5 mM MgCl <sub>2</sub> , 1 mM CaCl <sub>2</sub> , 0.5% BSA, 0.05% Tween
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 Orexin A (Bachem H-4172) at decreasing concentration (see graph below)
	25 µL of radioligand at the appropriate concentration (see graph below)
	150 µL of diluted membranes
3 - Incubation time:	30 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 % PEI).

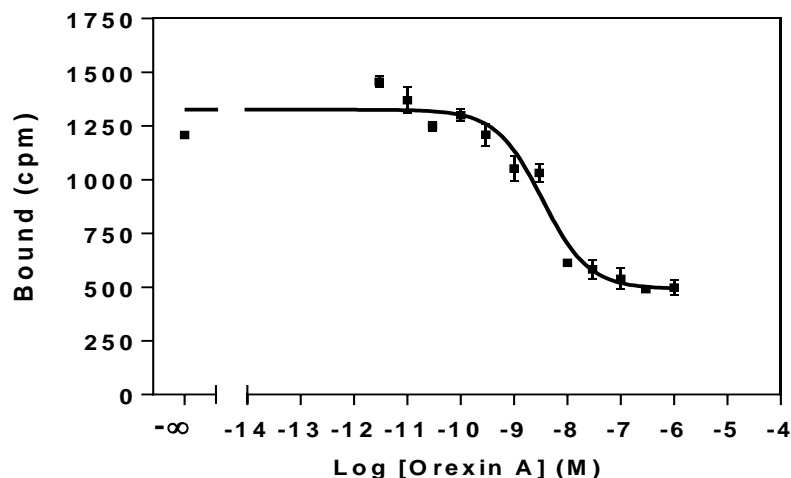


Figure 1: Competition binding assay curve (filtration) 96-well saturation binding assay curve (1  $\mu$ g membranes/well, TopCount<sup>®</sup>) using [<sup>125</sup>I]-Orexin A (Revvity NEX367 Lot No.: JB51550). Recommended radioligand concentration = 0.16 nM.

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

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