

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN OT OXYTOCIN RECEPTOR

CATALOG NUMBER:	HTS090M	QUANTITY:	200 units
LOT NUMBER:		VOLUME/CONCENTRATION:	1 mL, 1.0 mg/mL

BACKGROUND:

Oxytocin is a cyclic 9 amino acid peptide that differs from vasopressin in 2 amino acids. Despite the close similarities in sequence, oxytocin and vasopressin have different biological activities and bind to distinct G protein-coupled receptors. The oxytocin receptor, OT, couples primarily to $G_{q/11}$ to mobilize intracellular calcium. In female reproduction, oxytocin promotes uterine contraction and lactation; oxytocin is the most commonly used drug for induction of labor, whereas an oxytocin antagonist, atosiban, is under investigation to suppress preterm labor. Oxytocin/OT interaction in the CNS also plays an important role in stress, male and female sexual response, and sociality (Gimpl and Fahrenholz, 2001). Chemicon's oxytocin membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression; thus, they are ideal HTS tools for screening of antagonists of oxytocin receptor interactions with oxytocin. The membrane preparations exhibit a K_d of 0.53nM for [125 I]-OVTA. With 5.0 ug/well Oxytocin Membrane Prep and 0.5 nM [125 I]-OVTA, a greater than 6-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay, and GTP γ S binding.

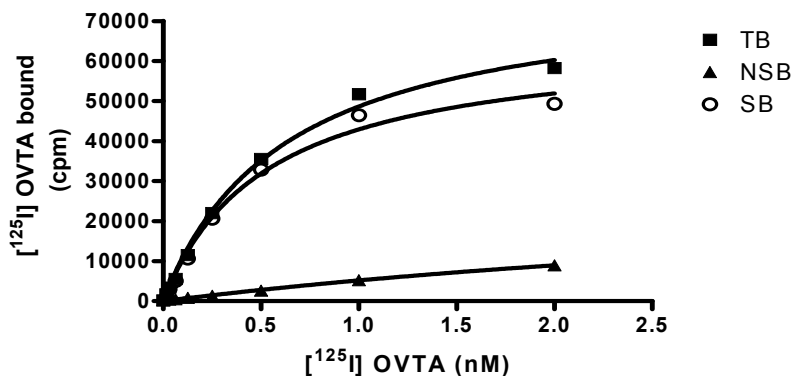


Figure 1. Saturation binding for Oxytocin Receptor. 5.0 μ g/well Oxytocin Membrane Preparation was incubated with increasing amount of [125 I]-OVTA in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled human recombinant Oxytocin receptor. Specific binding (SB) was determined by subtracting NSB from TB.

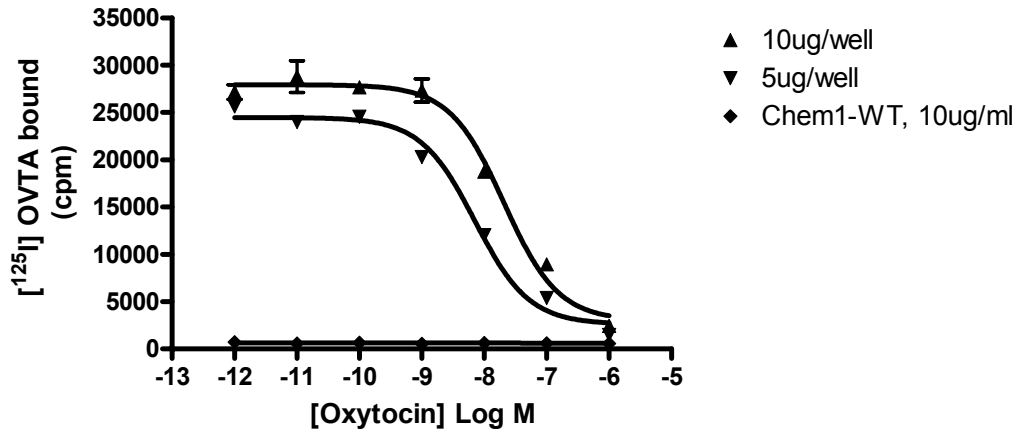


Figure 2. Competition binding for Oxytocin Receptor. Oxytocin Receptor Membrane Preparation (5.0 or 10µg/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 0.5 nM [¹²⁵I]-OVTA and increasing concentrations of unlabeled Oxytocin, and more than 6- fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of Oxytocin Receptor membrane prep.

	5 µg/well	10 µg/well
Signal:background	9	9
Specific binding (cpm)	21871	24926

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
 Bmax: 4.5 pmol/mg
 K_d: 0.53 nM

Species: Human Oxytocin Receptor (Accession number NM_000916)
 HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous Oxytocin Receptor expression.

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, a GF/C 96-well filter plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM HEPES, pH 7.4, 0.5% BSA. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 50 mM Hepes, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Radioligand: [¹²⁵I] OVTA (Perkin Elmer # NEX254)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl, 0.1% BSA, filtered and stored at 4°C.

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One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 6-fold signal:background with ^{125}I -labeled OVTA at 0.5 nM.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membranes protein was adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C .

STORAGE/HANDLING:

Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

REFERENCES:

Gimpl G and Fahrenholz F (2001) The oxytocin receptor system: structure, function and regulation. *Physiol. Rev.* 81: 629-683.

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Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 μL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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