

PRODUCT DATASHEET
ChemiScreen™ OT Oxytocin Membrane Preparation

CATALOG NUMBER:	HTS090M	QUANTITY:	200 units
LOT NUMBER:	SC20201210	VOLUME/CONCENTRATION:	1 mL, 1 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). The 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 0.17 nM for [¹²⁵I]-OVTA (ornithine vasotocin analogue). With 5 μg/well of Oxytocin Membrane Prep and 0.5 nM [¹²⁵I]-OVTA, a greater than 6-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand Binding Assay

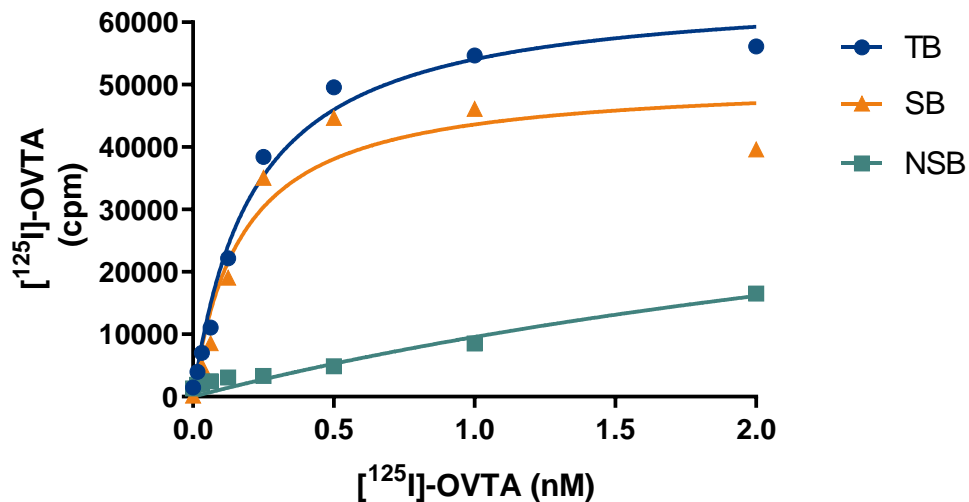


Figure 1. Saturation Binding for Oxytocin Receptor. 5 μg/well Oxytocin Receptor Membrane Preparation was incubated with increasing amounts of [¹²⁵I]-OVTA in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled human recombinant Oxytocin. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample lot.

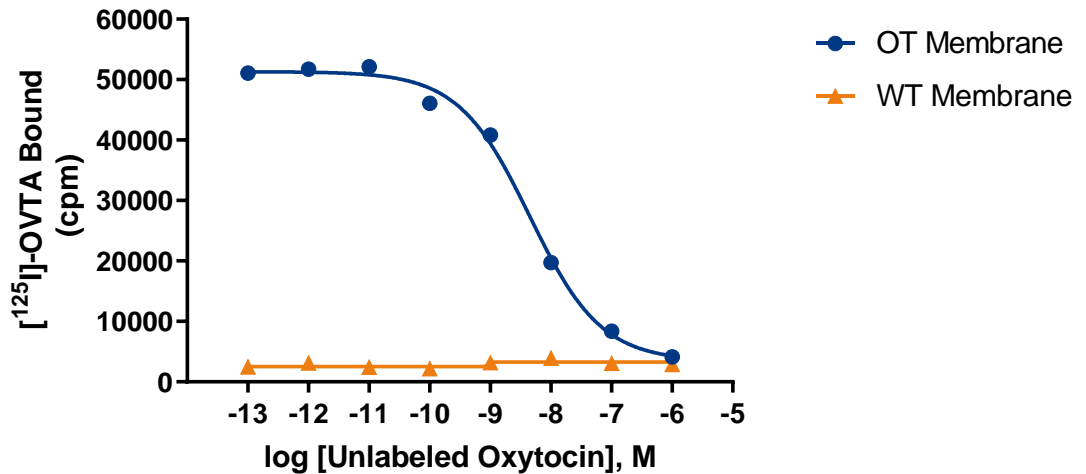


Figure 2. Competition Binding for Oxytocin Receptor. 5 μ g/well Oxytocin Receptor Membrane Preparation or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) were incubated with 0.5 nM [¹²⁵I]-OVTA and increasing concentrations of unlabeled Oxytocin, and more than 6-fold signal:background was obtained. The data are from a representative sample lot.

SPECIFICATIONS: 1 unit = 5 μ g
 B_{max} : 4.18 pmol/mg
 K_d : 0.17 nM
 Signal:background: >6-fold

Species: Human Oxytocin Receptor (Accession number NM_000916)

HOST CELLS: Chem-1, an adherent mammalian cell line with no detectable 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 2 h at room temperature. Prior to filtration, an FC 96-well harvest plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA at 200 μ L per well. The binding reactions are transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells are then 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 (PerkinElmer # NEX254)

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

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than a 6-fold signal:background ratio with [¹²⁵I]-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: Membrane proteins were adjusted to the indicated concentration in 1 mL packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Store at -70°C . Product is stable for at least 6 months from the date of receipt when stored as directed. Avoid repeated freeze/thaw cycles.

REFERENCES:

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

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

No part of these works may be reproduced in any form without permission in writing.

Eurofins Pharma Bioanalytics Services US Inc. is an independent member of Eurofins Discovery Services.