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PRODUCT DATASHEET

ChemiScreen™ Gonadotropin-Releasing Hormone Membrane Preparation

CATALOG NUMBER:	HTS027M	**QUANTITY:**	200 units
LOT NUMBER:	22C2403	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND: Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), regulates the reproductive hormonal cascade in vertebrates. Upon release from the hypothalamus, GnRH stimulates secretion of luteinizing hormone and follicle-stimulating hormone from the pituitary. In humans, the type I GnRH receptor is a GPCR that is unusual in the lack of a cytoplasmic C-terminal tail. Although some species contain a type II GnRH receptor, the human version of this second receptor appears to contain mutations that render it inactive. GnRH analogs (agonists) are used in low doses in a pulsatile fashion in the treatment of infertility, delayed puberty and cryptorchidism. GnRH agonists at high doses desensitize the receptor, and are used along with antagonists in the treatment of hormone-dependent diseases (Millar *et al.*, 2004). GnRH 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 agonists and antagonists at GnRH. The membrane preparations exhibit a Kd of 0.84 nM for [^{125}I]-[D-Trp^{6}]-LHRH. With 0.5 nM [^{125}I]-[D-Trp^{6}]-LHRH, 10 µg/well of GnRH Receptor Membrane Prep yields greater than a 3-fold signal-to-background ratio.

APPLICATIONS: Radioligand Binding Assay

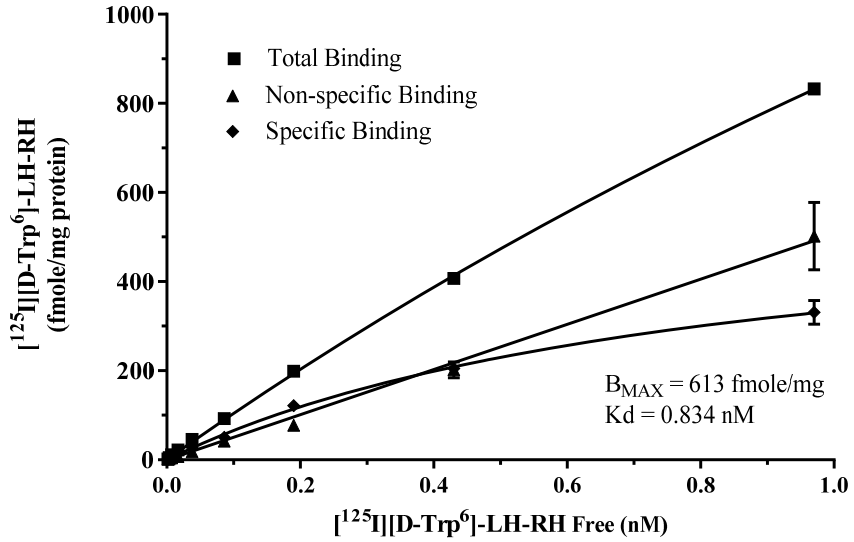

Figure 1. Saturation Binding for GnRH. 10 µg/well of GnRH Receptor Membrane Preparation was incubated with increasing amounts of [^{125} I]-LHRH in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled LHRH. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample lot.

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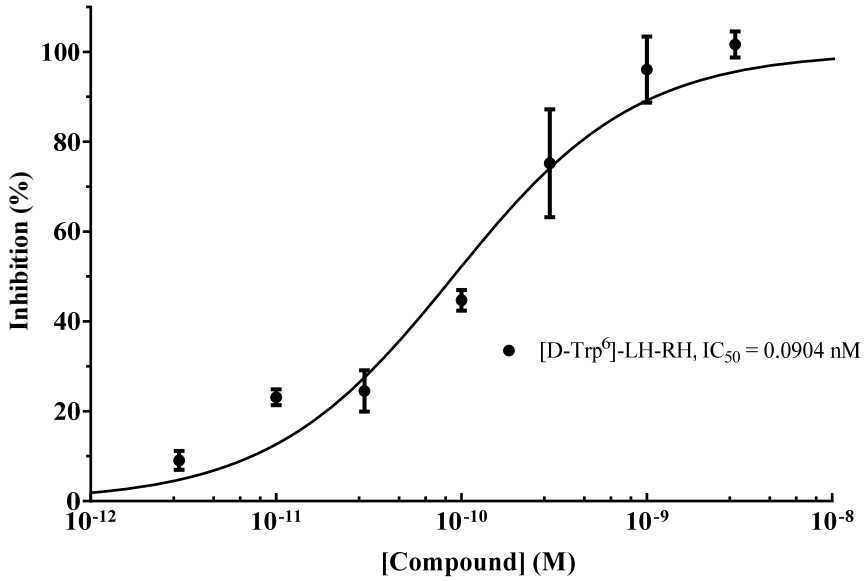

Figure 2. Competition Binding for GnRH. 10 µg/well GnRH Membrane Preparation was incubated with 0.5 nM [^{125}I]-LHRH and increasing concentrations of unlabeled LHRH, and more than a 3-fold signal:background ratio was obtained. The data are from a representative sample lot.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{125}I]-LHRH binding: 0.61 pmol/mg
K_d for [^{125}I]-LHRH binding: 0.83 nM
Signal:background: >3-fold

Species: Human Type I GnRH receptor (Accession number L03380)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous GnRH 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, a GF/C 96-well filter plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. 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 $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C.

Radioligand: [^{125}I]-[D-Trp^6]-LHRH (PerkinElmer # NEX365)

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 3-fold signal:background ratio with [^{125}I]-LHRH 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 indicated concentration in 1 mL packaging buffer, rapidly frozen, and stored at -80ºC.

STORAGE/HANDLING: Store at –80°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. Millar RP *et al.* (2004). Gonadotropin-releasing hormone receptors. *Endocr. Rev.* 25:235-275.

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