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PRODUCT DATASHEET

ChemiScreen™ GLP-1 Glucagon-Like Peptide Membrane Preparation

CATALOG NUMBER:	HTS163M	**QUANTITY:**	200 units
LOT NUMBER:	22M0715	**VOLUME/CONCENTRATION**	1 mL, 2 mg/mL

BACKGROUND: Glucagon-like peptide-I (GLP-1), part of the secretin peptide family, is a class B (class 2) receptor and is involved in glucose-dependent insulin secretion. Insulin secretion is predominantly controlled by glucose levels in the blood, however glucagon stimulates the secretion of insulin (Drucker *et al*. 1987). The GLP-1 receptor couples to G_s to increase cAMP levels (Mayo *et al*. 2003). GLP-1 has been shown to delay gastric emptying and regulate appetite. Due to the involvement in insulin secretion GLP-1 has been identified as a therapeutic target in type II diabetes (Mayo *et al*. 2003, D'Alessio *et al*. 2004). GLP-1 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 GLP-1 interactions with GLP-1.

APPLICATIONS: Radioligand binding assay and GTPγS binding

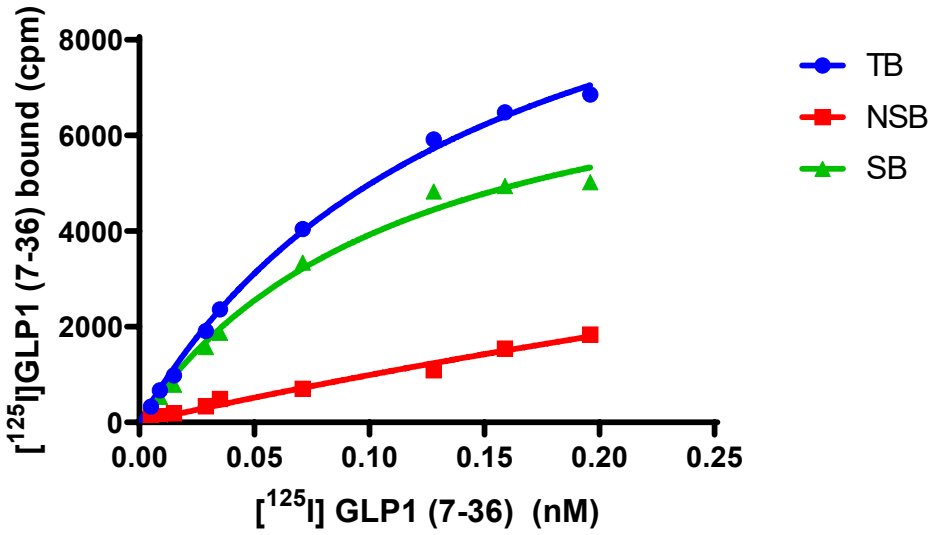

Figure 1. Saturation binding for GLP-1. 3 µg/well GLP-1 Membrane Preparation was incubated with increasing amount of [^{125}I]-GLP-1(7-36) in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled GLP-1(7-36). Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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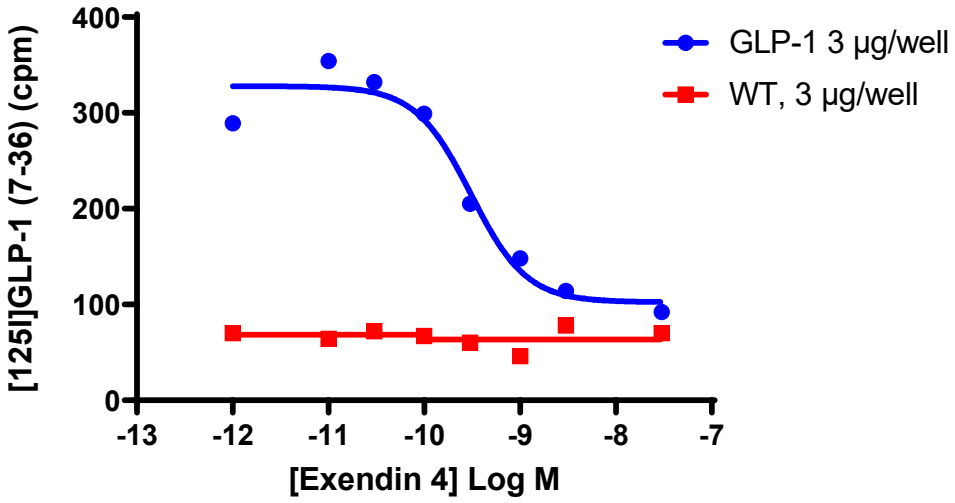

Figure 2. Competition binding for GLP-1. GLP-1 Membrane Preparation (3 µg/well) or Wild-Type membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.025 nM [^{125}I]-GLP-1(7-36) and increasing concentrations of unlabeled Exendin-4, and more than 3-fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 10 µg membrane preparation
Bmax: 0.83 pmol/mg
K_d: 0.117 nM
Signal:background: >3-fold

TRANSFECTION: Full-length human GLP-1 cDNA encoding GLP-1 (Accession Number: NM_002062).

HOST CELLS: Chem-9, an adherent cell line expressing the promiscuous G-protein, Gα15.

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, an FC 96-well harvest plate (Millipore cat. # MAHF C1H) 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 $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C

Radioligand: [^{125}I] GLP-1(7-36) (Perkin Elmer # NEX308)

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

One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 3-fold signal:background with ^{125}I-labeled GLP-1 at 0.025 nM.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membranes protein were adjusted to the indicated concentration in 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. Do not freeze and thaw.

REFERENCES:

1. Drucker *et al.* (1987) Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in rat islet cell line. *Proc. Natl. Acad. Sci.*, 84: 3434-3438
2. Mayo KE *et al.* (2003) International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacol. Rev.* 55: 167-194.
3. D'Alessio D *et al*. (2004) Glucagon-like peptide 1: evolution of an invretin into a treatment for diabetes. *Am. J. Physiol. Endocrinol. Metab*. 286: 882-890.