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CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN GLUCAGON RECEPTOR

CATALOG NUMBER:	HTS112M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION :**	1 mL, 1 mg/mL

BACKGROUND:

Glucagon is a 29-amino acid peptide that stimulates glycogenolysis and gluconeogenesis in the liver to increase blood glucose. The receptor for glucagon is a class 2 (or class B) GPCR that signals through G_s to stimulate cAMP production (Mayo *et al.*, 2003). Mice lacking the glucagon receptor have mild hypoglycemia after fasting, and exhibit hyperplasia of pancreatic α-cells (Gelling *et al.*, 2003). Because of its role in promoting hyperglycemia, the glucagon receptor presents a potential target for treatment of diabetes. Millipore's glucagon receptor 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 glucagon receptor interactions and its ligands. The membrane preparations exhibit a Kd of 3.09 nM for [^{125}I]-glucagon. With 1 nM [^{125}I]-glucagon, 5 µg/well Glucagon Receptor Membrane Prep yields greater than 10-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay

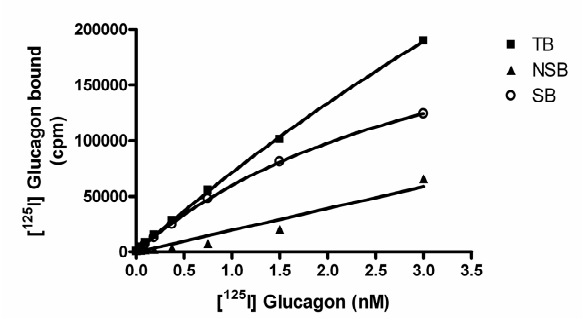

Figure 1. Saturation binding for Glucagon. 5 µg/well Glucagon Receptor Membrane Preparation was incubated with increasing amount of ^{125}I labeled glucagon in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled glucagon. Specific binding (SB) was determined by subtracting NSB from TB.

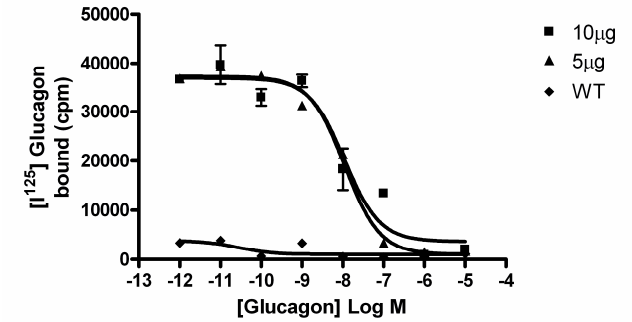

Figure 2. Competition binding for Glucagon. Glucagon Receptor Membrane Preparation (5 and 10 µg/well in a 96-well plate) were incubated with 1 nM ^{125}I labeled glucagon and increasing concentrations of unlabeled glucagon, and subjected to filtration binding.

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

	10 µg/well	5 µg/well
Signal:background	10.1	35
Specific binding (cpm)	33315	36252

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{125}I] glucagon binding: 16.7 pmol/mg protein;
K_d for [^{125}I] glucagon binding: ~ 3.09 nM

TRANSFECTION: Full-length human GCGR cDNA encoding Glucagon Receptor (Accession Number: NM_000160)

HOST CELLS: Chem-1, an adherent mammalian cell line with no endogenous glucagon 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, an FC 96-well harvest plate (Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM 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℃

Radioligand: [^{125}I]-glucagon (Perkin Elmer#:NEX-207)

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

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 10-fold signal:background with ^{125}I labeled glucagon at 1 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℃. Product is stable for at least 6 m onths from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

Gelling RW *et al.* (2003) Lower blood glucose, hyperglucagonemia, and pancreatic α cell hyperplasia in glucagon receptor knockout mice. *Proc. Natl. Aca. Sci. USA* 100:1438-1443.

Mayo KE *et al.* (2003) International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacol. Rev.* 55: 167-194.

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06/20/08/HTS112M/ML/LA