



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. Chemicon'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 K_d of 3.09 nM for [125 I]-glucagon. With 1 nM [125 I]-glucagon, 5 or 10 μ g/well glucagon receptor Membrane Prep yields greater than 10-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

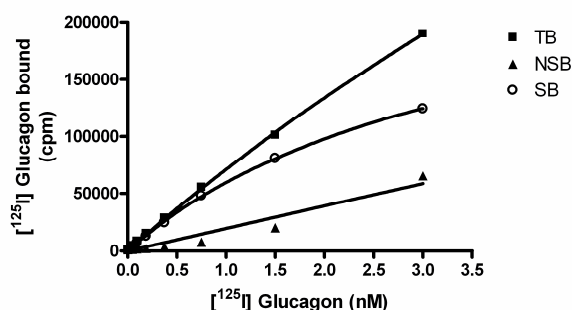


Figure 1. Saturation binding for Glucagon. 5 μ g/well glucagon receptor Membrane Preparation was incubated with increasing amount of [125 I] labeled glucagon in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled glucagon. Specific binding (SB) was determined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

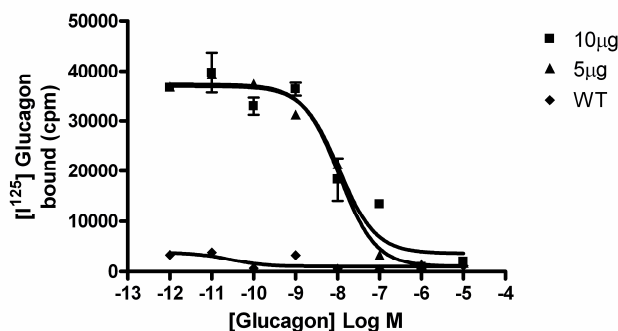


Figure 2. Competition binding for Glucagon. Glucagon receptor Membrane Preparation (5 and 10 µg/well in a 96-well plate) were incubated with 1 nM ¹²⁵I labeled glucagon and increasing concentrations of unlabeled glucagon, and subjected to filtration binding.

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 [¹²⁵I] glucagon binding: 16.7 pmol/mg protein;
 K_d for [¹²⁵I] 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, 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: [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°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 10-fold signal:background with 125 I labeled glucagon at 1 nM.

PRESENTATION:

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 3 H-labeled MK-912 at 5 nM

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 1 mg/mL in packaging buffer, and dispensed at 1 mL/vial. Vials were 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:

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.

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 μ L 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.

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

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