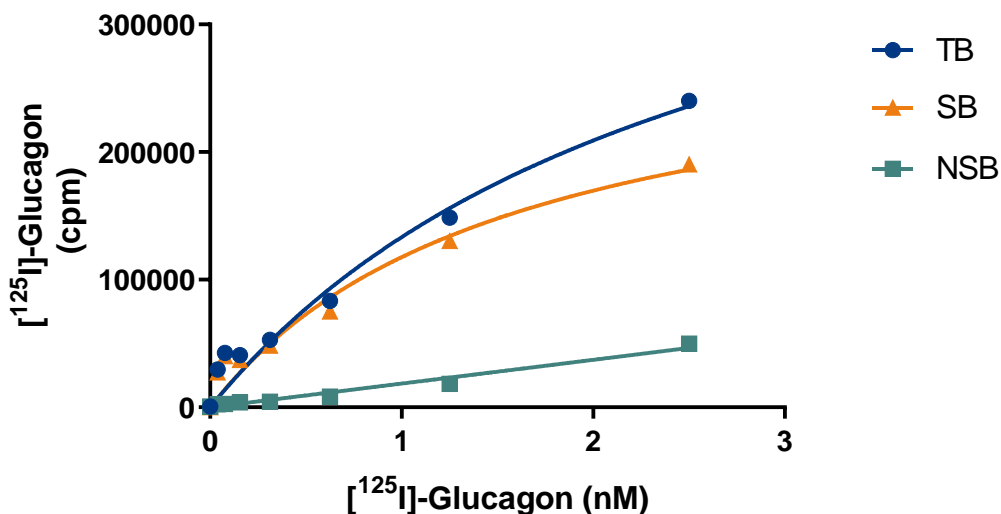


**PRODUCT DATASHEET**
**ChemiScreen™ Glucagon Receptor Membrane Preparation**

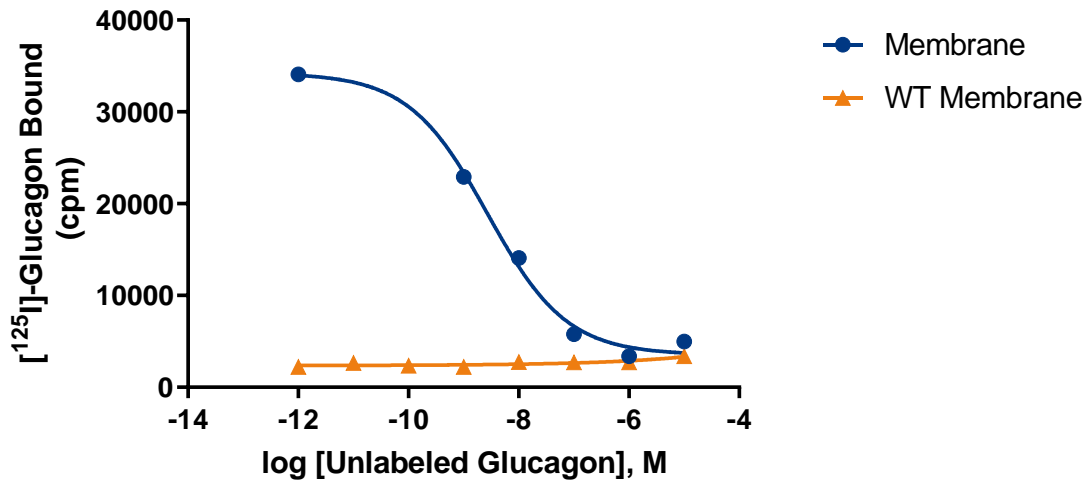
<b>CATALOG NUMBER:</b>	HTS112M	<b>QUANTITY:</b>	200 units
<b>LOT NUMBER:</b>	SC20201223	<b>VOLUME/CONCENTRATION:</b>	1 mL, 1 mg/mL

**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  $\alpha$ -cells (Gelling *et al.*, 2003). Because of its role in promoting hyperglycemia, the glucagon receptor presents a potential target for treatment of diabetes. 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 0.64 nM for [ $^{125}$ I]-Glucagon.

**APPLICATIONS:** Radioligand Binding Assay



**Figure 1. Saturation Binding for Glucagon.** 5  $\mu$ g/well Glucagon Receptor Membrane Preparation was incubated with increasing amounts of [ $^{125}$ I]-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. The data are from a representative sample of lot SC20201223.



**Figure 2. Competition Binding for Glucagon.** 5 µg/well of Glucagon Receptor Membrane Preparation or wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 1 nM [<sup>125</sup>I]-Glucagon and increasing concentrations of unlabeled glucagon. The data are from a representative sample of lot SC20201223.

**SPECIFICATIONS:** 1 unit = 5 µg  
B<sub>max</sub> for [<sup>125</sup>I]-Glucagon Binding: 14.77 pmol/mg protein  
K<sub>d</sub> for [<sup>125</sup>I]-Glucagon Binding: 0.64 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 were mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a non-binding 96-well plate, and incubated for 2 h. Prior to filtration, a GF/C 96-well filter plate was coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reactions were transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells were dried and counted.

**Binding Buffer:** 50 mM Hepes, pH 7.4, 5 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.2% BSA, filtered and stored at 4°C

**Radioligand:** [<sup>125</sup>I]-Glucagon (PerkinElmer#: NEX207 )

**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 a 4-fold signal:background ratio with [<sup>125</sup>I]-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: Membrane proteins were adjusted to 1 mg/mL in packaging buffer and dispensed at 1 mL/vial. The 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. Avoid repeated freeze/thaw cycles.

**REFERENCES:**

1. 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.
2. Mayo KE *et al.* (2003). International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacol. Rev.* 55:167-194.

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