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PRODUCT DATASHEET

ChemiScreen™ GIP Glucagon Family Receptor Membrane Preparation

CATALOG NUMBER:	HTS134M	**QUANTITY:**	200 units
LOT NUMBER:	SC20161129	**VOLUME/CONCENTRATION:**	2 mL, 1 mg/mL

BACKGROUND: Gastric inhibitory polypeptide (GIP), also known as glucose-dependent insulinotropic polypeptide, is a 42 amino acid peptide that is secreted from the duodenum and proximal jejunum after food intake. The receptor for GIP is a family 2 GPCR that is most closely related to the glucagon and GLP-1 receptors, and like these receptors it couples to Gs to stimulate intracellular cAMP (Mayo *et al.*, 2003). The GIP receptor is most highly expressed in pancreas, and the primary physiological function of GIP is to potentiate glucose-mediated secretion of insulin (Yip *et al.*, 1999). The GIP receptor is expressed in many other tissues, and accordingly GIP also has extrapancreatic activities. GIP secreted after meal ingestion stimulates bone formation, and mice lacking the GIP receptor display features of osteoporosis (Tsukiyama *et al.*, 2006). In addition, GIP increases insulin sensitivity in adipose tissue. Since patients with type 2 diabetes display decreased responsiveness to GIP, a defect in GIP receptor expression and/or signaling may lead to β-cell dysfunction and type 2 diabetes (Meier and Nauck, 2004). GIP 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 agonists and antagonists at GIP Receptor. The membrane preparations exhibit a Kd of 0.18 nM for [^{125}I]-GIP. With 0.2 nM [^{125}I]-GIP, GIP Receptor Membrane Prep typically yields greater than 5-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

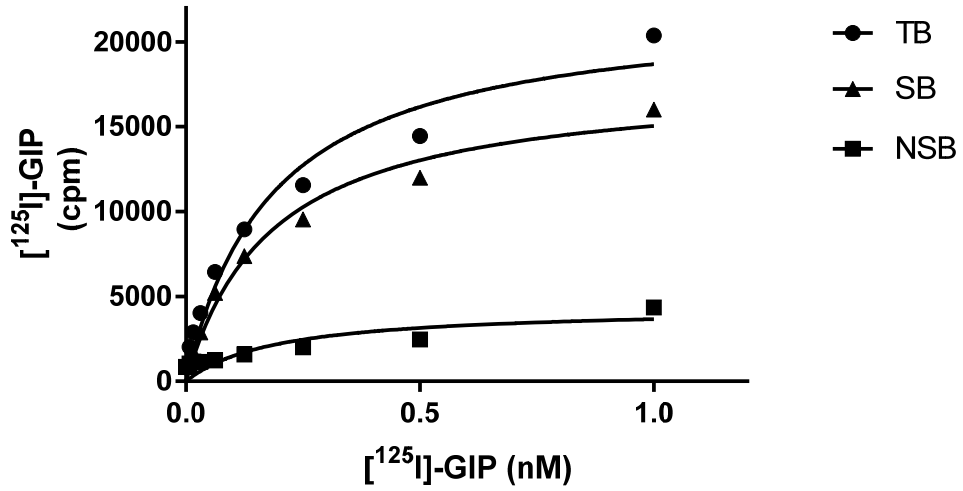

Figure 1. Saturation binding for GIP Receptor. GIP Receptor Membrane Preparation was incubated with increasing amount of ^{125}I-labeled GIP in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 1000-fold excess unlabeled GIP. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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Template version: 7. Modified Date: 05DED16. Modified by: JVK

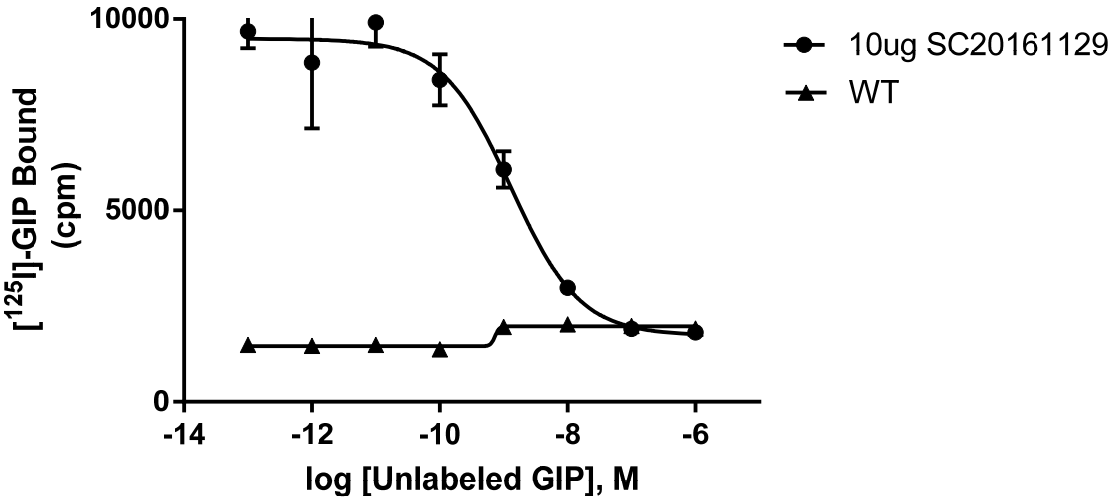

Figure 2. Competition binding for GIP Receptor. GIP Receptor Membrane Preparation and wild-type Chem-1 Membrane Preparation were incubated in a 96-well plate with 0.2 nM ^{125}I-labeled GIP and increasing concentrations of unlabeled GIP. More than 5- fold signal:background was obtained with GIP. Representative sample data.

SPECIFICATIONS: B_{max}: 0.73 pmol/mg protein
K_d: ~0.18 nM
Signal:background: >5-fold

TRANSFECTION: Full-length human GIPR cDNA encoding GIP Receptor (Accession Number: NM_000164)

Species: Human

HOST CELLS: Chem-9, an adherent mammalian cell line with minimum amount of endogenous GIP 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 (EMD 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°C

Radioligand: [^{125}I] GIP (Perkin Elmer#**:** NEX-402)

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 5-fold signal:background with ^{125}I-labeled GIP at 0.2 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 the indicated concentration in 1 ml 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. Mayo KE *et al.* (2003). International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacol. Rev.* 55: 167-194.
2. Meier JJ and Nauck MA (2004) GIP as a potential therapeutic agent? *Horm. Metab. Res.* 36(11-12): 859-866
3. Tsukiyama K *et al.* (2006). Gastric Inhibitory Polypeptide as an Endogenous Factor Promoting New Bone Formation after Food Ingestion. *Mol. Endocrin.* 20(7): 1644-1651.
4. Yip RGC *et al.* (1999). GIP Biology and Fat Metabolism. *Life Sci.* 66(2): 91-103.