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**READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS**
**HUMAN RECOMBINANT GIP RECEPTOR, GLUCAGON RECEPTOR FAMILY**

| | | | |
|---|---|---|---|
| **CATALOG NUMBER:** | HTS134F | **QUANTITY:** | 1 vial, 1 mL |
| **LOT NUMBER:** | | **CONCENTRATION:** | 1 x $10^7$ viable cells/mL |

**BACKGROUND:**

Millipore's Ready-To-Assay™ Calcium-Optimized Cells are GPCR-expressing cell lines that are designed for simple, rapid calcium assays with no requirement for culturing cells. The user simply thaws the cells with maximal viability, dispenses into assay plates, and assays for calcium response the next day.

The Ready-To-Assay™ cells are derived from ChemiScreen™ calcium-optimized stable cell lines, which express the GPCR target of interest at high levels on the cell surface, in a host cell line containing high levels of the promiscuous Gα15 protein to couple the receptor to the calcium signaling pathway. The Ready-To-Assay™ cells are prepared by chemical treatment at a concentration optimized for effective growth arrest while maintaining high viability (>80%) after thawing and overnight plating. Pharmacological functionality of the Ready-To-Assay cells is identical to that of the originating GPCR cell line.

Gastric inhibitory polypeptide receptor (GIP) has been identified in the glucose-mediated secretion of insulin(Mayo *et al.*, 2003). GIP is in the secretin/VIP receptor family which includes secretin, VIP, glucagon, GLP-1, growth hormone releasing hormone (GHRH), and PACAP (Yip *et al.*, 1999). GIP is secreted after meal ingestion has been shown to stimulate bone formation resulting in lower occurrences of osteoporosis (Tsukiyama *et al.*, 2006). Type 2 diabetes is a result of decreased glucose-stimulated insulin secretion which makes insulin secretion potentiators a popular target for diabetes treatments; a defect in GIP expression and/or signaling may lead to β-cell dysfunction and type 2 diabetes (Mayo *et al.*, 2003). Millipore's cloned human GIP receptor-expressing cell line is made in the Chem-9 host, an adherent cell line that supports high levels of recombinant GIP receptor expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. The untreated GIP receptor-Chem-9 cell line and the Ready-To-Assay™ GIP receptor cells have equivalent EC50s for GIP.

**APPLICATIONS:**

Calcium flux assay

SPECIFICATIONS:

| | EC50 for GIP (nM) | Maximum Signal (RFU) | Z' |
|---|---|---|---|
| Ready-To-Assay Cells | 5.4 | 2202 | 0.73 |
| Continuous Passage Cells | 5.7 | 3138 | 0.72 |

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

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

PLATING MEDIA:
DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
100 U/mL Pen-Strep (from 100x stock, Millipore TMS-AB2-C)

**PRESENTATION:**

Cells are frozen at 1 x $10^7$ cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO.

**STORAGE:**

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years.

**ASSAY PROTOCOL:**

1) Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.

2) Transfer contents of the vial to a sterile 15 mL conical tube. Add 10 mL prewarmed plating media to the cells and mix gently to resuspend cells. Centrifuge at 200 x g. Remove all but 0.5 mL media.

3) Resuspend cells to 0.5 x $10^6$ cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200 µL per well to obtain a density of approximately 1 x $10^5$ cells/well.

4) Place the assay plate in a humidified 37°C incubator with 5% $CO_2$.

5) The cells may be assayed 16-24 hours after plating. It is recommended to wash the cells with assay buffer at least once prior to addition of loading dye.

**REFERENCES:**

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

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.

Yip RGC *et al.* (1999). GIP Biology and Fat Metabolism. *Life Sci.* 66(2): 91-103.

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**HUMAN RECOMBINANT GIP GLUCAGON FAMILY RECEPTOR**
**Product No. HTS134F**

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