

Ready-to-Assay™ Motilin Receptor Frozen Cells

HTS121RTA

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Product Overview

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. We have optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

Motilin is a 22-amino acid peptide that potently stimulates gastrointestinal contractility. The biological effects of motilin are mediated by a G_q-coupled seven transmembrane protein, currently termed motilin receptor (MR), that shares significant sequence similarity with the ghrelin receptor.¹ The motilin receptor is also activated by the antibiotic erythromycin; this interaction appears to mediate some of the gastrointestinal side effects of erythromycin. Although motilides (non-antibiotic derivatives of erythromycin) such as ABT-229 have been investigated for treatment of diabetic gastroparesis, their effectiveness has been limited by tachyphylaxis (decreased response to ligand) resulting from receptor downregulation.² Agonists of the motilin receptor with reduced desensitization activity remain a potential treatment for disorders of gastric motility. Our cloned human motilin-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant Motilin expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists, and modulators of the motilin receptor.

Materials Provided

- Pack contains 2 vials of mycoplasma-free cells, 1 mL per vial, store in liquid N₂.
- Fifty (50) mL of Media Component store at 4 °C (-20 °C for prolonged storage).

Storage and Stability

Vials are to be stored in liquid N₂. Media Component at 4 °C (-20 °C for prolonged storage).

Use Restrictions

Please see User Agreement (Label License) for further details. One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.

Applications

Calcium Flux Assays

Application Data

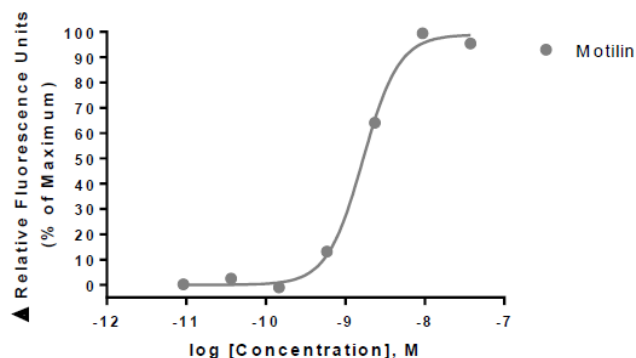


Figure 1. Representative data for activation of motilin receptor. Calcium flux in motilin-expressing Chem-1 cell line induced by motilin. Motilin-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPRTETRA. Maximal fluorescence signal obtained in this experiment was 7,000 RLU (Relative Light Units).

Table I. EC₅₀ values of Motilin-expressing Chem-1 cells.

Ligand	Assay	Potency (nM)	Reference
Motilin	Calcium Flux	1.7	Internal Data

Assay Setup

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37 °C water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1 mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes.
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37 °C 5% CO₂ incubator for 24 hours.
9. After 24-hour incubation, remove assay plate from the incubator and invert plate to remove Media Component.
10. Prepare Fluo-8 NW (ABD Bioquest 21080) Ca²⁺ dye by dissolving 1 mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca²⁺ dye solution into 10 mL of HBSS 20 mM HEPES, 2.5 mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca²⁺ dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm (FLIPR^{TETRA}) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR^{TETRA}) or emission filter for Ca²⁺ dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning® plates (Corning® 3605, 96-well or Corning® 3574, 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

Host Cell

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Ga15 protein.

Exogenous Gene Expression

MLNR cDNA (Accession Number: NM_001507) expressed from a proprietary plasmid.

Related Products

1. HTSCHEM-1RTA - Ready-to-Assay™ Chem-1 host frozen cells (control cells).
2. HTS121M - ChemiScreen™ Motilin Receptor membrane prep.

References

1. Feighner SD et al. (1999) Receptor for motilin identified in the human gastrointestinal system. Science 284: 2184-2188.
2. Thielemans L. et al. (2005) Desensitization of the human motilin receptor by motilides. J. Pharmacol. Exp. Ther. 313: 1397-405.

GMO

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