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PRODUCT DATASHEET

Ready-to-Assay™ MC_5 Melanocortin Receptor Frozen Cells

CATALOG NUMBER: HTS155RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has 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.

The melanocortin system consists of five seven-transmembrane spanning G-protein coupled receptors, MC_1-MC_5 (Gantz and Fong, 2003). Among the five members of the melanocortin receptor family, MC_2 and MC_5 are expressed in peripheral tissues. The MC_2 receptor (ACTH receptor) is almost exclusively expressed in the adrenal cortex whereas MC_5 is expressed in several organs including the adrenal cortex. Studies have shown that targeted disruption of the MC5R gene produced mice with a severe defect in water repulsion and thermoregulation caused by decreased production of sebaceous lipids (Chen *et al.*, 1997). In addition, MC_5 is required for secretion from several exocrine glands, indicating that melanocortin peptides integrate the function of exocrine glands (Entwistle *et al.,* 1990). Cloned human MC_5 -expressing cell line is made in the Chem-10 host, which supports high levels of recombinant MC_5 expression on the cell surface and contains optimized levels of a recombinant promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists, and modulators at MC_5.

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.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T | +1 844 522 7787
F | +1 636 362 7131
www.eurofins.com

APPLICATIONS

Calcium Flux Assays, cAMP Accumulation

APPLICATION DATA

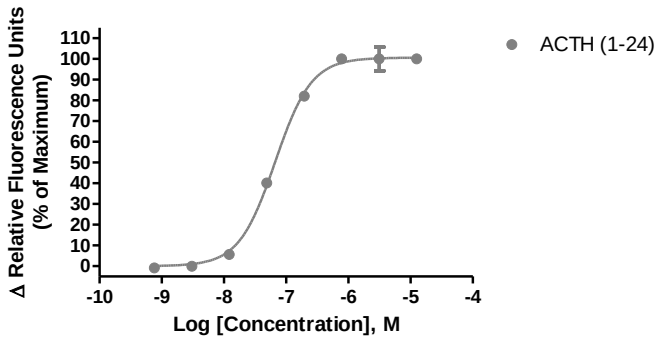

Figure 1. Representative data for activation of MC_5 receptor. Calcium flux in MC_5–expressing Chem-10 cell line induced by Adrenocorticotropin (ACTH 1-24). MC_5–expressing Chem-10 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 1,900 RLU (Relative Light Units).

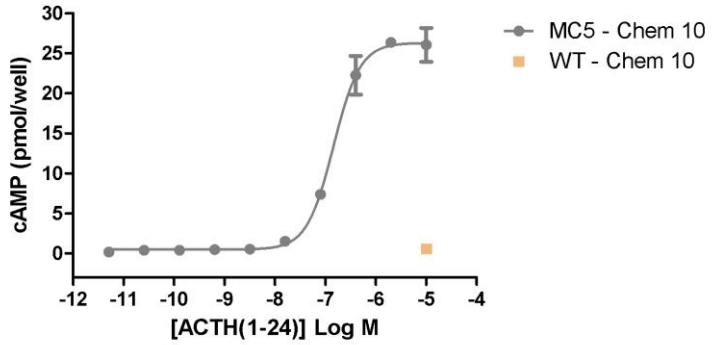

Figure 2. Representative data for activation of MC_5 receptor stably expressed in CHEM-10 cells induced by ACTH(1-24) using a cAMP accumulation assay. MC_5–expressing CHEM-10 cells were seeded at 100,000 cells per well into a 96-well plate, and the following day the cells were treated with ACTH(1-24) for 15 minutes in the presence of 2.0 mM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal cAMP response obtained in this experiment was 27 pmol/well. Similarly parental cells (catalog #: HTSCHEM-10) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of MC_5-expressing Chem-10 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
ACTH (1-24)	Calcium Flux	67	Eurofins Internal Data
ACTH (1-24)	cAMP Accumulation	144	Eurofins 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 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL 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 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.
10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} 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 Ca^{2+} 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.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
ACTH (1-24) ligand	Bachem: H1150
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-10, an adherent rat hematopoietic cell line expressing endogenous G‧15 protein as well as an exogenous proprietary promiscuous Gα protein.

EXONGENOUS GENE EXPRESSION

MC5R cDNA (Accession Number: NM_005913; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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ATG AAT TCC TCA TTT CAC CTC CAT TTC TTG GAT CTC AAC CTG AAT GCC ACA GAG GGC AAC CTT TCA GGA
 M   N   S   S   F   H   L   H   F   L   D   L   N   L   N   A   T   E   G   N   L   S   G

CCC AAT GTC AAA AAC AAG TCT TCA CCA TGT GAA GAC ATG GGC ATT GCT GTG GAG GTG TTT CTC ACT CTG
 P   N   V   K   N   K   S   S   P   C   E   D   M   G   I   A   V   E   V   F   L   T   L

GGT GTC ATC AGC CTC TTG GAG AAC ATC TTG GTC ATA GGG GCC ATA GTG AAG AAC AAA AAC CTG CAC TCC
 G   V   I   S   L   L   E   N   I   L   V   I   G   A   I   V   K   N   K   N   L   H   S

CCC ATG TAC TTC TTC GTG TGC AGC CTG GCA GTG GCG GAC ATG CTG GTG AGC ATG TCC AGT GCC TGG GAG
 P   M   Y   F   F   V   C   S   L   A   V   A   D   M   L   V   S   M   S   S   A   W   E

ACC ATC ACC ATC TAC CTA CTC AAC AAC AAG CAC CTA GTG ATA GCA GAC GCC TTT GTG CGC CAC ATT GAC
 T   I   T   I   Y   L   L   N   N   K   H   L   V   I   A   D   A   F   V   R   H   I   D

AAT GTG TTT GAC TCC ATG ATC TGC ATT TCC GTG GTG GCA TCC ATG TGC AGC TTA CTG GCC ATT GCA GTG
 N   V   F   D   S   M   I   C   I   S   V   V   A   S   M   C   S   L   L   A   I   A   V

GAT AGG TAC GTC ACC ATC TTC TAC GCC CTG CGC TAC CAC CAC ATC ATG ACG GCG AGG CGC TCA GGG GCC
 D   R   Y   V   T   I   F   Y   A   L   R   Y   H   H   I   M   T   A   R   R   S   G   A

ATC ATC GCC GGC ATC TGG GCT TTC TGC ACG GGC TGC GGC ATT GTC TTC ATC CTG TAC TCA GAA TCC ACC
 I   I   A   G   I   W   A   F   C   T   G   C   G   I   V   F   I   L   Y   S   E   S   T

TAC GTC ATC CTG TGC CTC ATC TCC ATG TTC TTC GCT ATG CTG TTC CTC CTG GTG TCT CTG TAC ATA CAC
 Y   V   I   L   C   L   I   S   M   F   F   A   M   L   F   L   L   V   S   L   Y   I   H

ATG TTC CTC CTG GCG CGG ACT CAC GTC AAG CGG ATC GCG GCT CTG CCC GGG GCC AGC TCT GCG CGG CAG
 M   F   L   L   A   R   T   H   V   K   R   I   A   A   L   P   G   A   S   S   A   R   Q

AGG ACC AGC ATG CAG GGC GCG GTC ACC GTC ACC ATG CTG CTG GGC GTG TTT ACC GTG TGC TGG GCC CCG
 R   T   S   M   Q   G   A   V   T   V   T   M   L   L   G   V   F   T   V   C   W   A   P
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TTC TTC CTT CAT CTC ACT TTA ATG CTT TCT TGC CCT CAG AAC CTC TAC TGC TCT CGC TTC ATG TCT CAC
 F   F   L   H   L   T   L   M   L   S   C   P   Q   N   L   Y   C   S   R   F   M   S   H

TTC AAT ATG TAC CTC ATA CTC ATC ATG TGT AAT TCC GTG ATG GAC CCT CTC ATA TAT GCC TTC CGC AGC
 F   N   M   Y   L   I   L   I   M   C   N   S   V   M   D   P   L   I   Y   A   F   R   S

CAA GAG ATG CGG AAG ACC TTT AAG GAG ATT ATT TGC TGC CGT GGT TTC AGG ATC GCC TGC AGC TTT CCC
 Q   E   M   R   K   T   F   K   E   I   I   C   C   R   G   F   R   I   A   C   S   F   P

AGA AGG GAT TGA
 R   R   D  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS155M	ChemiScreen™ MC_5 Melanocortin receptor membrane prep

Note: Chem-10 cells are derived from Chem-1 cells.

REFERENCES

1. Chen W *et al.* (1997) Exocrine gland dysfunction in MC5-R-deficient mice: evidence for coordinated regulation of exocrine gland function by melanocortin peptides. *Cell* 91: 789-798.
2. Entwistle ML *et al.* (1990) Characterization of functional melanotropin receptors in lacrimal glands of the rat. *Peptides* 11: 477-483.
3. Gantz I and Fong TM (2003) The melanocortin system. *Am. J. Physiol. Endocrinol. Metab.* 284: E468-E474.

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