eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ MC_2 Melanocortin Receptor Frozen Cells

CATALOG NUMBER: HTS021RTA

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 melanocortins, α-, β- and γ-melanocyte-stimulating hormones (MSHs) and adrenocorticotropin (ACTH), are peptides derived from a precursor protein POMC. The MSH peptides and ACTH bind to a family of five Class 1 Gs-coupled seven transmembrane receptors (MC1-5) and play important roles in energy balance, reproductive function, pigmentation and inflammation (Gantz and Fong, 2003). MC_2, the ACTH receptor, is a member of this family but is the only one that does not bind the MSHs, it instead binds ACTH exclusively. MC_2 is expressed mainly in cells of the adrenal cortex, where it signals cells in the adrenal cortex to synthesize and secrete glucocorticoids (Clark A. et al., 2006). Mutations in MC_2 lead to familial glucocorticoid deficiency, or ACTH insensitivity. Familial glucocorticoid deficiency can lead to increased pigmentation and increased longitudinal bone growth (Clark A, et al. 1993, Imamine H, et al. 2005). In addition to mutations in MC_2 leading to Familial glucocorticod deficiency, it has also been shown that mutations in a protein called MC2-R accessory protein (MRAP) also lead to this disorder (Clark A, 2005). Expression of MC_2 on the cell surface has been found to be dependent on the interaction of MC2 with MRAP. This interaction helps to move MC_2 from the endoplasmic reticulum to the cell surface. Cloned human MC2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant MC_2 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 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_2.

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.

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

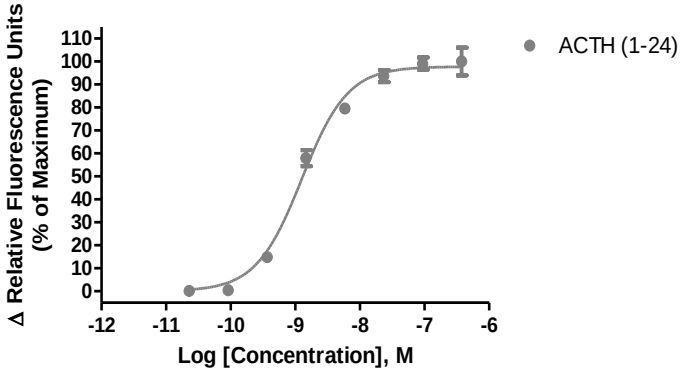

Figure 1. Representative data for activation of MC_2 receptor. Calcium flux in MC_2–expressing Chem-1 cell line induced by Adrenocorticotropin (ACTH 1-24). MC_2–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 14,810 RLU (Relative Light Units).

Table 1. EC_{50} value of MC_2-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
ACTH (1-24)	Calcium Flux	1.3	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-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein

EXONGENOUS GENE EXPRESSION

Human MC2R cDNA (Accession Number: NM_000529) and MRAP (Accession Number: NM_178817) expressed from a proprietary plasmid.

RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)

REFERENCES

1. Gantz I and Fong TM (2003) The melanocortin system. *Am. J. Physiol. Endocrinol. Metab.* 284: E468-E474.
2. Clark A and Metherell L (2006) Mechanisms of Disease: the adrenocorticotropin receptor and disease. *Nature* 2(5):282-90. Review
3. Clark A *et al*. (1993) Familial glucocorticoid deficiency associated with point mutation in the adrenocorticotropin receptor. *Lancet* 341: 461-462.
4. Imamine H *et al*. (2005) Possible relationship between elevated plasma ACTH and tall stature in familial glucocorticoid deficiency. *Tohoku J Exp Med* 205: 123-131.
5. Clark A *et al*. (2005) Inherited ACTH insensitivity illuminates the mechanisms of ACTH action. *Trends Endocrinol Metab* 16(10): 451-457.

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