

PRODUCT DATASHEET

Ready-to-Assay™ MCH₁ Melanin-Concentrating Hormone Receptor Frozen Cells

CATALOG NUMBER: HTS025RTA

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 N₂. Media Component at 4°C (-20°C 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.

[Melanin-concentrating hormone (MCH) is a cyclic, 19 amino acid peptide produced in the lateral hypothalamus and other regions of the mammalian brain (Bittencourt *et al.*, 1992). When administered intracerebrally or overexpressed transgenically in rodents, MCH stimulates food intake and weight gain (Ludwig *et al.*, 2001). A receptor for MCH, previously identified as an orphan receptor SLC-1 and renamed MCH₁, has been identified in humans and rodents. Consistent with a role in hypothalamic control of feeding behavior, MCH₁ is expressed in the ventromedial and dorsomedial nuclei of the hypothalamus (Chambers *et al.*, 1999). Genetic ablation and pharmacological antagonism of MCH₁ in rodents results in resistance to obesity caused by a high fat diet or leptin deficiency (Borowsky *et al.*, 2002; Segal-Lieberman, *et al.*, 2003; Shearman *et al.*, 2003; Marsh *et al.*, 2002; Chen *et al.*, 2002). Thus, MCH₁ is an attractive target for obesity. Cloned human MCH₁-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant MCH₁ 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 at MCH₁.

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.

APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

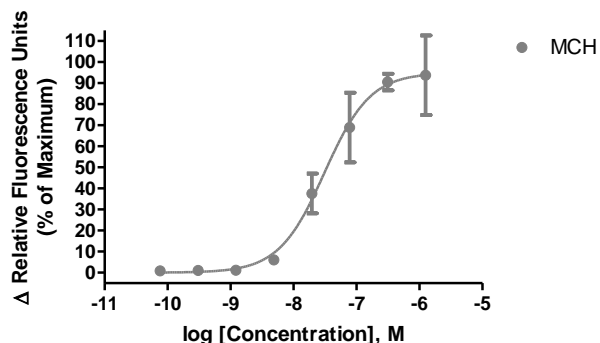


Figure 1. Representative data for activation of MCH₁ receptor. Calcium flux in MCH₁-expressing Chem-1 cell line induced by Melanin-Concentrating Hormone (MCH). MCH₁-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution 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 24,000 RLU (Relative Light Units).

Table 1. EC₅₀ value of MCH₁-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
MCH	Calcium Flux	31	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 37°C 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 37°C 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) Ca²⁺ dye by dissolving 1mg 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 20mM HEPES, 2.5mM 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.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenecid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
MCH ligand	Sigma: M4542
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

MCHR1 cDNA (Accession Number: NM_005297; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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208 - ATG GAC CTG GAA GCC TCG - 225
70 - M D L E A S - 75

CTG CTG CCC ACT GGT CCC AAC GCC AGC AAC ACC TCT GAT GGC CCC GAT AAC CTC ACT TCG GCA GGA TCA CCT CCT - 300
L L P T G P N A S N T S D G P D N L T S A G S P P - 100

CGC ACG GGG AGC ATC TCC TAC ATC AAC ATC ATC ATG CCT TCG GTG TTC GGC ACC ATC TGC CTC CTG GGC ATC ATC - 375
R T G S I S Y I N I I M P S V F G T I C L L G I I - 125

GGG AAC TCC ACG GTC ATC TTC GCG GTC GTG AAG AAG TCC AAG CTG CAC TGG TGC AAC AAC GTC CCC GAC ATC TTC - 450
G N S T V I F A V V K K S K L H W C N N V P D I F - 150

ATC ATC AAC CTC TCG GTA GTA GAT CTC CTC TTT CTC CTG GGC ATG CCC TTC ATG ATC CAC CAG CTC ATG GGC AAT - 525
I I N L S V V D L L F L L G M P F M I H Q L M G N - 175

GGG GTG TGG CAC TTT GGG GAG ACC ATG TGC ACC CTC ATC ACG GCC ATG GAT GCC AAT AGT CAG TTC ACC AGC ACC - 600
G V W H F G E T M C T L I T A M D A N S Q F T S T - 200

TAC ATC CTG ACC GCC ATG GCC ATT GAC CGC TAC CTG GCC ACT GTC CAC CCC ATC TCT TCC ACG AAG TTC CGG AAG - 675
Y I L T A M A I D R Y L A T V H P I S S T K F R K - 225

CCC TCT GTG GCC ACC CTG GTG ATC TGC CTC CTG TGG GCC CTC TCC TTC ATC AGC ATC ACC CCT GTG TGG CTG TAT - 750
P S V A T L V I C L L W A L S F I S I T P V W L Y - 250

GCC AGA CTC ATC CCC TTC CCA GGA GGT GCA GTG GGC TGC GGC ATA CGC CTG CCC AAC CCA GAC ACT GAC CTC TAC - 825
A R L I P F P G G A V G C G I R L P N P D T D L Y - 275

TGG TTC ACC CTG TAC CAG TTT TTC CTG GCC TTT GCC CTG CCT TTT GTG GTC ATC ACA GCC GCA TAC GTG AGG ATC - 900
W F T L Y Q F F L A F A L P F V V I T A A Y V R I - 300

CTG CAG CGC ATG ACG TCC TCA GTG GCC CCC GCC TCC CAG CGC AGC ATC CGG CTG CGG ACA AAG AGG GTG ACC CGC - 975
L Q R M T S S V A P A S Q R S I R L R T K R V T R - 325

ACA GCC ATC GCC ATC TGT CTG GTC TTC TTT GTG TGC TGG GCA CCC TAC TAT GTG CTA CAG CTG ACC CAG TTG TCC - 1050
T A I A I C L V F F V C W A P Y Y V L Q L T Q L S - 350

ATC AGC CGC CCG ACC CTC ACC TTT GTC TAC TTA TAC AAT GCG GCC ATC AGC TTG GGC TAT GCC AAC AGC TGC CTC - 1125
I S R P T L T F V Y L Y N A A I S L G Y A N S C L - 375

AAC CCC TTT GTG TAC ATC GTG CTC TGT GAG ACG TTC CGC AAA CGC TTG GTC CTG TCG GTG AAG CCT GCA GCC CAG - 1200
N P F V Y I V L C E T F R K R L V L S V K P A A Q - 400

GGG CAG CTT CGC GCT GTC AGC AAC GCT CAG ACG GCT GAC GAG GAG AGG ACA GAA AGC AAA GGC ACC TGA - 1269
G Q L R A V S N A Q T A D E E R T E S K G T Stp - 425

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RELATED PRODUCTS
PRODUCT NUMBER
DESCRIPTION

HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS025M	ChemiScreen™ MCH ₁ Melanin-Concentrating Hormone receptor membrane prep

REFERENCES

1. Bittencourt J.C., *et al.* (1992) The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. *J. Comp. Neurol.* 319: 218-45.
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3. Chambers J., *et al.* (1999) Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1. *Nature* 400: 261-5.
4. Chen Y. *et al.* (2002) Targeted disruption of the melanin-concentrating hormone receptor-1 results in hyperphagia and resistance to diet-induced obesity. *Endocrinology* 143:2469-77.
5. Ludwig D.S. *et al.* (2001) Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *J. Clin. Invest.* 107:379-86.
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7. Segal-Lieberman G. *et al.* (2003) Melanin-concentrating hormone is a critical mediator of the leptin-deficient phenotype. *Proc. Natl. Acad. Sci. USA* 100:10085-90.
8. Shearman L.P. *et al.* (2003) Chronic MCH-1 receptor modulation alters appetite, body weight and adiposity in rats. *Eur. J. Pharmacol.* 475:37-47.

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