

PRODUCT DATASHEET

Ready-to-Assay™ 5-HT₆ Serotonin Receptor Frozen Cells

CATALOG NUMBER: HTS111RTA

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 over night recovery, assays for calcium response.

The neurotransmitter serotonin/5-hydroxytryptamine (5-HT) regulates a wide variety of neurological functions. A family of 13 receptors (12 GPCRs and one ion channel) mediates the effects of serotonin. The serotonin receptor 5-HT₆ is a G_s coupled receptor expressed solely in the CNS, primarily in the limbic and cortical regions. 5-HT₆ appears to play a role in memory and learning, obesity, psychosis, anxiety and epilepsy (Woolley et al., 2004; Fisas et al., 2006). In particular, a 5-HT₆-selective agonist caused significant weight loss in a rat model of diet-induced obesity. Cloned human 5-HT₆-expressing cell line is made in the Chem-10 host, which supports high levels of recombinant 5-HT₆ 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 5-HT₆.

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

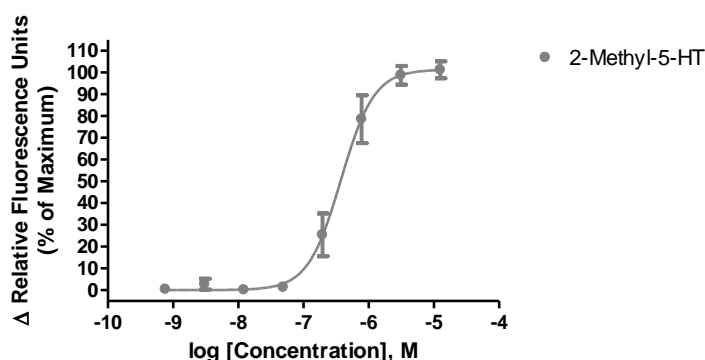


Figure 1. Representative data for activation of 5-HT₆ receptor. Calcium flux in 5-HT₆-expressing Chem-10 cell line induced by 2-methyl-5-HT. 5-HT₆-expressing Chem-10 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 3,100 RLU (Relative Light Units).

Table 1. EC₅₀ value of 5-HT₆-expressing Chem-10 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
2-methyl-5-HT	Calcium Flux	380	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 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. dissolved place 10 µL of Fluo-8 NW Ca²⁺ dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7 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 474-495 nm (FLIPR^{TETRA}) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR^{TETRA}) or 515-565 nm (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense

to 75 $\mu\text{L}/\text{sec}$ (96-well format) or 50 $\mu\text{L}/\text{sec}$ (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 sta

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH3026802
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
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 \cdot 15 protein as well as an exogenous proprietary promiscuous G α protein.

EXONGENOUS GENE EXPRESSION

HTR6 cDNA (Accession Number: NM_000871; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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ATG GTC CCA GAG CCG GGC CCA ACC GCC AAT AGC ACC
M V P E P G P T A N S T

CCG GCC TGG GGG GCA GGG CCG CCG TCG GCC CCG GGG GGC AGC GGC TGG GTG GCG GCC GCG CTG TGC GTG
P A W G A G P P S A P G G S G W V A A A L C V

GTC ATC GCG CTG ACG GCG GCG GCC AAC TCG CTG CTG ATC GCG CTC ATC TGC ACT CAG CCC GCG CTG CGC
V I A L T A A A N S L L I A L I C T Q P A L R

AAC ACG TCC AAC TTC TTC CTG GTG TCG CTC TTC ACG TCT GAC CTG ATG GTG GGG CTG GTG GTG ATG CCG
N T S N F F L V S L F T S D L M V G L V V M P

CCG GCC ATG CTG AAC GCG CTG TAC GGG CGC TGG GTG CTG GCG CGC GGC CTC TGC CTG CTC TGG ACC GCC
P A M L N A L Y G R W V L A R G L C L L W T A

TTC GAC GTG ATG TGC TGC AGC GCC TCC ATC CTC AAC CTC TGC CTC ATC AGC CTG GAC CGC TAC CTG CTC
F D V M C C S A S I L N L C L I S L D R Y L L

ATC CTC TCG CCG CTG GCG TAC AAG CTG CGC ATG ACG CCC CTG CGT GCC CTG GCC CTA GTC CTG GGC GCC
I L S P L R Y K L R M T P L R A L A L V L G A

TGG AGC CTC GCC GCT CTC GCC TCC TTC CTG CCC CTG CTG CTG GGC TGG CAC GAG CTG GGC CAC GCA CGG
W S L A A L A S F L P L L L G W H E L G H A R

CCA CCC GTC CCT GGC CAG TGC CGC CTG CTG GTC AGC CTG CCT TTT GTC CTT GTG GCG TCG GGC CTC ACC
P P V P G Q C R L L V S L P F V L V A S G L T

TTC TTC CTG CCC TCG GGT GCC ATA TGC TTC ACC TAC TGC AGG ATC CTG CTA GCT GCC CGC AAG CAG GCC
F F L P S G A I C F T Y C R I L L A A R K Q A

GTG CAG GTG GCC TCC CTC ACC ACC GGC ATG GCC AGT CAG GCC TCG GAG ACG CTG CAG GTG CCC AGG ACC
V Q V A S L T T G M A S Q A S E T L Q V P R T

CCA CGC CCA GGG GTG GAG TCT GCT GAC AGC AGG CGT CTA GCC ACG AAG CAC AGC AGG AAG GCC CTG AAG
P R P G V E S A D S R R L A T K H S R K A L K

GCC AGC CTG ACG CTG GGC ATC CTG CTG GGC ATG TTC TTT GTG ACC TGG TTG CCC TTC TTT GTG GCC AAC
A S L T L G I L L G M F F V T W L P F F V A N

ATA GTC CAG GCC GTG TGC GAC TGC ATC TCC CCA GGC CTC TTC GAT GTC CTC ACA TGG CTG GGT TAC TGT
I V Q A V C D C I S P G L F D V L T W L G Y C

AAC AGC ACC ATG AAC CCC ATC ATC TAC CCA CTC TTC ATG CGG GAC TTC AAG CGG GCG CTG GGC AGG TTC
N S T M N P I I Y P L F M R D F K R A L G R F

CTG CCA TGT CCA CGC TGT CCC CGG GAG CGC CAG GCC AGC CTG GCC TCG CCA TCA CTG CGC ACC TCT CAC
L P C P R C P R E R Q A S L A S P S L R T S H

AGC GGC CCC CGG CCC GGC CTT AGC CTA CAG CAG GTG CTG CCG CTG CCC CTG CCG CCG GAC TCA GAT TCG
S G P R P G L S L Q Q V L P L P L P P D S D S

GAC TCA GAC GCA GGC TCA GGC GGC TCC TCG GGC CTG CGG CTC ACG GCC CAG CTG CTG CTT CCT GGC GAG
D S D A G S G G S S G L R L T A Q L L L P G E

GCC ACC CAG GAC CCC CCG CTG CCC ACC AGG GCC GCT GCC GCC GTC AAT TTC TTC AAC ATC GAC CCC GCG
A T Q D P P L P T R A A A A V N F F N I D P A

GAG CCC GAG CTG CGG CCG CAT CCA CTT GGC ATC CCC ACG AAC TGA
E P E L R P H P L G I P T N Stp

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RELATED PRODUCTS
PRODUCT NUMBER
DESCRIPTION
HTSCHEM-1RTA*
Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS111M
ChemiScreen™ 5-HT6 serotonin family receptor membrane prep

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

REFERENCES

1. Boess FG *et al.* (1997) Functional and radioligand binding characterization of rat 5-HT₆ receptors stably expressed in HEK293 cells. *Neuropharmacology* 36: 713-720.
2. Fisas A *et al.* (2006) Chronic 5-HT₆ receptor modulation by E-6837 induces hypophagia and sustained weight loss in diet-induced obese rats. *Br. J. Pharmacol.* 148: 973-83.
3. Woolley ML *et al.* (2004) 5-HT₆ receptors. *Curr. Drug Targets CNS Neurol. Disord.* 3: 59-79.

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