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PRODUCT DATASHEET

Ready-to-Assay™ 5-HT$_{4B}$ Serotonin Receptor Frozen Cells

CATALOG NUMBER: HTS110RTA

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.

5-Hydroxytryptamine (5-HT, also commonly known as serotonin) is synthesized in enterochromaffin cells in the intestine and in serotonergic nerve terminals. In the periphery, 5-HT mediates gastrointestinal motility, platelet aggregation, and contraction of blood vessels. Many functions of the central nervous system are influenced by 5-HT, including sleep, motor activity, sensory perception, arousal and appetite. A family of 12 GPCRs and one ion channel mediate the biological effects of 5-HT (Hoyer *et al.*, 1994). 5-HT$_4$ comprises at least 8 isoforms varying at the C-terminus, which are generated by alternative splicing. The expression and distribution of these splice variants differs among organs and tissues with many of them present in several tissues such as atrium, brain, and GI tract (Bockaert *et al.* 2004). To date, all isoforms have been shown to activate adenylyl cyclase *in vitro*, and no difference in signal transduction between C-terminal 5-HT$_4$ receptor variants has been demonstrated. 5-HT$_4$ receptors are of potential interest for the treatment of patients with GI motility disorders and Alzheimer's disease (De Maeyer *et al.* 2008; Lezoualc'h 2007). Cloned human 5HT$_{4B}$-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant 5HT$_{4B}$ 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 5HT$_{4B}$ Receptor.

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

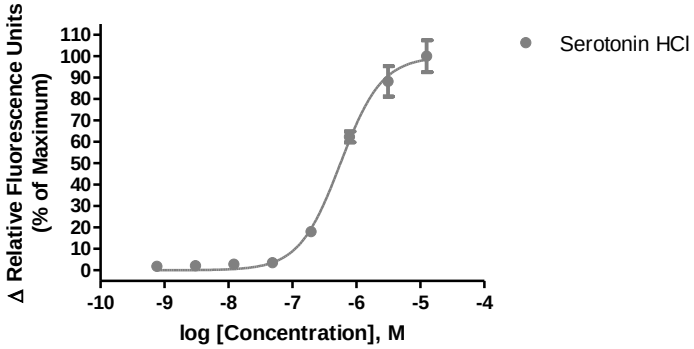

Figure 1. Representative data for activation of 5-HT_{4B} receptor. Calcium flux in 5-HT_{4B} –expressing Chem-1 cell line induced by Serotonin. 5-HT_{4B} –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 1,000 RLU (Relative Light Units).

Table 1. EC_{50} values of $5HT_{4B}$ -expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Serotonin	Calcium Flux	560	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
Serotonin ligand	Sigma: H9523
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

HTR4 cDNA (Accession Number: NM_000870; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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ATG GAC AAA CTT GAT GCT AAT GTG AGT TCT GAG GAG GGT TTC GGG TCA GTG GAG AAG GTG GTG CTG     66
 M   D   K   L   D   A   N   V   S   S   E   E   G   F   G   S   V   E   K   V   V   L      22

CTC ACG TTT CTC TCG ACG GTT ATC CTG ATG GCC ATC TTG GGG AAC CTG CTG GTG ATG GTG GCT GTG    132
 L   T   F   L   S   T   V   I   L   M   A   I   L   G   N   L   L   V   M   V   A   V      44

TGC TGG GAC AGG CAG CTC AGG AAA ATA AAA ACA AAT TAT TTC ATT GTA TCT CTT GCT TTT GCG GAT    198
 C   W   D   R   Q   L   R   K   I   K   T   N   Y   F   I   V   S   L   A   F   A   D      66

CTG CTG GTT TCG GTG CTG GTG ATG CCC TTT GGT GCC ATT GAG CTG GTT CAA GAC ATC TGG ATT TAT    264
 L   L   V   S   V   L   V   M   P   F   G   A   I   E   L   V   Q   D   I   W   I   Y      88

GGG GAG GTG TTT TGT CTT GTT CGG ACA TCT CTG GAC GTC CTG CTC ACA ACG GCA TCG ATT TTT CAC    330
 G   E   V   F   C   L   V   R   T   S   L   D   V   L   L   T   T   A   S   I   F   H     110

CTG TGC TGC ATT TCT CTG GAT AGG TAT TAC GCC ATC TGC TGC CAG CCT TTG GTC TAT AGG AAC AAG    396
 L   C   C   I   S   L   D   R   Y   Y   A   I   C   C   Q   P   L   V   Y   R   N   K     132

ATG ACC CCT CTG CGC ATC GCA TTA ATG CTG GGA GGC TGC TGG GTC ATC CCC ACG TTT ATT TCT TTT    462
 M   T   P   L   R   I   A   L   M   L   G   G   C   W   V   I   P   T   F   I   S   F     154

CTC CCT ATA ATG CAA GGC TGG AAT AAC ATT GGC ATA ATT GAT TTG ATA GAA AAG AGG AAG TTC AAC    528
 L   P   I   M   Q   G   W   N   N   I   G   I   I   D   L   I   E   K   R   K   F   N     176

CAG AAC TCT AAC TCT ACG TAC TGT GTC TTC ATG GTC AAC AAG CCC TAC GCC ATC ACC TGC TCT GTG    594
 Q   N   S   N   S   T   Y   C   V   F   M   V   N   K   P   Y   A   I   T   C   S   V     198

GTG GCC TTC TAC ATC CCA TTT CTC CTC ATG GTG CTG GCC TAT TAC CGC ATC TAT GTC ACA GCT AAG    660
 V   A   F   Y   I   P   F   L   L   M   V   L   A   Y   Y   R   I   Y   V   T   A   K     220

GAG CAT GCC CAT CAG ATC CAG ATG TTA CAA CGG GCA GGA GCC TCC TCC GAG AGC AGG CCT CAG TCG    726
 E   H   A   H   Q   I   Q   M   L   Q   R   A   G   A   S   S   E   S   R   P   Q   S     242

GCA GAC CAG CAT AGC ACT CAT CGC ATG AGG ACA GAG ACC AAA GCA GCC AAG ACC CTG TGC ATC ATC    792
 A   D   Q   H   S   T   H   R   M   R   T   E   T   K   A   A   K   T   L   C   I   I     264

ATG GGT TGC TTC TGC CTC TGC TGG GCA CCA TTC TTT GTC ACC AAT ATT GTG GAT CCT TTC ATA GAC    858
 M   G   C   F   C   L   C   W   A   P   F   F   V   T   N   I   V   D   P   F   I   D     286

TAC ACT GTC CCT GGG CAG GTG TGG ACT GCT TTC CTC TGG CTC GGC TAT ATC AAT TCC GGG TTG AAC    924
 Y   T   V   P   G   Q   V   W   T   A   F   L   W   L   G   Y   I   N   S   G   L   N     308

CCT TTT CTC TAC GCC TTC TTG AAT AAG TCT TTT AGA CGT GCC TTC CTC ATC ATC CTC TGC TGT GAT    990
 P   F   L   Y   A   F   L   N   K   S   F   R   R   A   F   L   I   I   L   C   C   D     330

GAT GAG CGC TAC CGA AGA CCT TCC ATT CTG GGC CAG ACT GTC CCT TGT TCA ACC ACA ACC ATT AAT   1056
 D   E   R   Y   R   R   P   S   I   L   G   Q   T   V   P   C   S   T   T   T   I   N     352

GGA TCC ACA CAT GTA CTA AGG GAT GCA GTG GAG TGT GGT GGC CAG TGG GAG AGT CAG TGT CAC CCG   1122
 G   S   T   H   V   L   R   D   A   V   E   C   G   G   Q   W   E   S   Q   C   H   P     374

CCA GCA ACT TCT CCT TTG GTG GCT GCT CAG CCC AGT GAC ACT TAG TGA
 P   A   T   S   P   L   V   A   A   Q   P   S   D   T   *  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS110M	ChemiScreen™ $5HT_4$ Serotonin Receptor membrane prep

REFERENCES

1. Bockaert J *et al.* (2004) 5-HT_4 receptors. *Curr. Drug Targets CNS Neurol. Disord.* 3: 39-51.
2. De Maeyer JH *et al.* (2008) 5-HT_4 receptor agonists: similar but not the same. *Neurogastroenterol. Motil.* 20: 99-112.
3. Hoyer D *et al.* 1994 International Union of Pharmacology classification of receptors for 5-HT hydroxytryptamin (Serotonin). *Pharmacol. Rev.* 46: 157-203.
4. Lezoualc'h F (2007) 5-HT4 receptor and Alzheimer's disease: the amyloid connection. *Exp. Neurol.* 205: 325-329.
5. Pindon A *et al.* (2002) Differences in signal transduction of two 5-HT_4 receptor splice variants: compound specificity and dual coupling with Gαs- and Gαi/o-proteins. *Mol. Pharmacol.* 61: 85-96.
6. Vickery RG *et al.* (2007) A comparison of the pharmacological properties of guinea-pig and human recombinant 5-HT_4 receptors. *Br. J. Pharmacol.* 150: 782-791.

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