eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ 5-HT$_{2C}$ Serotonin Family Receptor Frozen Cells

CATALOG NUMBER: HTS132RTA

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 over night 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$_{2C}$, which couples to Gq in most cells to stimulate intracellular calcium, is prominently expressed in brain and appears to modulate depression, anxiety and appetite (Miller, 2005; Serretti et al., 2004; Wood, 2003). The mRNA encoding 5-HT$_{2C}$ undergoes selective RNA editing that changes 4 amino acids in the second intracellular loop; these changes result in alteration of efficiency of coupling to G proteins. Alterations in editing of 5-HT$_{2C}$ have been detected in victims of suicidal depression and in mice treated with the SSRI, fluoxetine (Tohda et al., 2006). Cloned human 5-HT$_{2C}$ -expressing cell line is made in the Chem-1 host, which supports high levels of recombinant 5-HT$_{2C}$ 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 5-HT$_{2C}$.

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

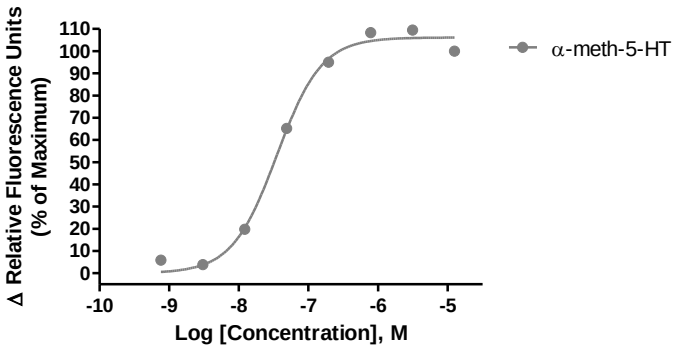

Figure 1. Representative data for activation of 5-HT_{2C} receptor. Calcium flux in 5-HT_{2C} –expressing Chem-1 cell line induced by α-meth-5-HT. 5-HT_{2C} –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 7000 RLU (Relative Light Units).

Table 1. EC_{50} value of $5HT_{2C}$-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
α-meth-5-HT	Calcium Flux	35	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
α-meth-5-HT ligand	Tocris: 0557
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

HTR2C cDNA (Accession Number: NM_000868; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG GTG AAC TTG AGG AAT GCG GTG CAT TCA TTC CTT
                                                 M   V   N   L   R   N   A   V   H   S   F   L

GTG CAC CTA ATT GGC CTA TTG GTT TGG CAA TGT GAT ATT TCT GTG AGC CCA GTA GCA GCT ATA GTA ACT
 V   H   L   I   G   L   L   V   W   Q   C   D   I   S   V   S   P   V   A   A   I   V   T

GAC ATT TTC AAT ACC TCC GAT GGT GGA CGC TTC AAA TTC CCA GAC GGG GTA CAA AAC TGG CCA GCA CTT
 D   I   F   N   T   S   D   G   G   R   F   K   F   P   D   G   V   Q   N   W   P   A   L

TCA ATC GTC ATC ATA ATA ATC ATG ACA ATA GGT GGC AAC ATC CTT GTG ATC ATG GCA GTA AGC ATG GAA
 S   I   V   I   I   I   I   M   T   I   G   G   N   I   L   V   I   M   A   V   S   M   E

AAG AAA CTG CAC AAT GCC ACC AAT TAC TTC TTA ATG TCC CTA GCC ATT GCT GAT ATG CTA GTG GGA CTA
 K   K   L   H   N   A   T   N   Y   F   L   M   S   L   A   I   A   D   M   L   V   G   L

CTT GTC ATG CCC CTG TCT CTC CTG GCA ATC CTT TAT GAT TAT GTC TGG CCA CTA CCT AGA TAT TTG TGC
 L   V   M   P   L   S   L   L   A   I   L   Y   D   Y   V   W   P   L   P   R   Y   L   C

CCC GTC TGG ATT TCT TTA GAT GTT TTA TTT TCA ACA GCG TCC ATC ATG CAC CTC TGC GCT ATA TCG CTG
 P   V   W   I   S   L   D   V   L   F   S   T   A   S   I   M   H   L   C   A   I   S   L

GAT CGG TAT GTA GCA ATA CGT AAT CCT ATT GAG CAT AGC CGT TTC AAT TCG CGG ACT AAG GCC ATC ATG
 D   R   Y   V   A   I   R   N   P   I   E   H   S   R   F   N   S   R   T   K   A   I   M

AAG ATT GCT ATT GTT TGG GCA ATT TCT ATA GGT GTA TCA GTT CCT ATC CCT GTG ATT GGA CTG AGG GAC
 K   I   A   I   V   W   A   I   S   I   G   V   S   V   P   I   P   V   I   G   L   R   D

GAA GAA AAG GTG TTC GTG AAC AAC ACG ACG TGC GTG CTC AAC GAC CCA AAT TTC GTT CTT ATT GGG TCC
 E   E   K   V   F   V   N   N   T   T   C   V   L   N   D   P   N   F   V   L   I   G   S

TTC GTA GCT TTC TTC ATA CCG CTG ACG ATT ATG GTG ATT ACG TAT TGC CTG ACC ATC TAC GTT CTG CGC
 F   V   A   F   F   I   P   L   T   I   M   V   I   T   Y   C   L   T   I   Y   V   L   R

CGA CAA GCT TTG ATG TTA CTG CAC GGC CAC ACC GAG GAA CCG CCT GGA CTA AGT CTG GAT TTC CTG AAG
 R   Q   A   L   M   L   L   H   G   H   T   E   E   P   P   G   L   S   L   D   F   L   K

TGC TGC AAG AGG AAT ACG GCC GAG GAA GAG AAC TCT GCA AAC CCT AAC CAA GAC CAG AAC GCA CGC CGA
 C   C   K   R   N   T   A   E   E   E   N   S   A   N   P   N   Q   D   Q   N   A   R   R

AGA AAG AAG AAG GAG AGA CGT CCT AGG GGC ACC ATG CAG GCT ATC AAC AAT GAA AGA AAA GCT TCG AAA
 R   K   K   K   E   R   R   P   R   G   T   M   Q   A   I   N   N   E   R   K   A   S   K

GTC CTT GGG ATT GTT TTC TTT GTG TTT CTG ATC ATG TGG TGC CCA TTT TTC ATT ACC AAT ATT CTG TCT
 V   L   G   I   V   F   F   V   F   L   I   M   W   C   P   F   F   I   T   N   I   L   S

GTT CTT TGT GAG AAG TCC TGT AAC CAA AAG CTC ATG GAA AAG CTT CTG AAT GTG TTT GTT TGG ATT GGC
 V   L   C   E   K   S   C   N   Q   K   L   M   E   K   L   L   N   V   F   V   W   I   G

TAT GTT TGT TCA GGA ATC AAT CCT CTG GTG TAT ACT CTG TTC AAC AAA ATT TAC CGA AGG GCA TTC TCC
 Y   V   C   S   G   I   N   P   L   V   Y   T   L   F   N   K   I   Y   R   R   A   F   S

AAC TAT TTG CGT TGC AAT TAT AAG GTA GAG AAA AAG CCT CCT GTC AGG CAG ATT CCA AGA GTT GCC GCC
 N   Y   L   R   C   N   Y   K   V   E   K   K   P   P   V   R   Q   I   P   R   V   A   A

ACT GCT TTG TCT GGG AGG GAG CTT AAT GTT AAC ATT TAT CGG CAT ACC AAT GAA CCG GTG ATC GAG AAA
 T   A   L   S   G   R   E   L   N   V   N   I   Y   R   H   T   N   E   P   V   I   E   K

GCC AGT GAC AAT GAG CCC GGT ATA GAG ATG CAA GTT GAG AAT TTA GAG TTA CCA GTA AAT CCC TCC AGT
 A   S   D   N   E   P   G   I   E   M   Q   V   E   N   L   E   L   P   V   N   P   S   S

GTG GTT AGC GAA AGG ATT ACG AGT GTG TGA
 V   V   S   E   R   I   T   S   V  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS132M	ChemiScreen™ 5-HT_{2C} serotonin family receptor membrane prep

REFERENCES

1. Barnes NM and Sharp T (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38, 1083-1152.
2. Miller KJ (2005) Serotonin 5-HT_{2C} receptor agonists: potential for the treatment of obesity. *Mol. Interv.* 5: 282-91.
3. Serretti A *et al.* (2004) The 5-HT_{2C} receptor as a target for mood disorders. *Expert Opin. Ther. Targets* 8: 15-23.
4. Tohda M *et al.* (2006) The molecular pathopharmacology of 5-HT_{2C} receptors and the RNA editing in the brain. *J. Pharmacol. Sci.* 100: 427-432.
5. Wood (2003) Therapeutic potential of 5-HT_{2C} receptor antagonists in the treatment of anxiety disorders. *Curr. Drug Targets CNS Neurol. Disord.* 2: 383-7.

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