

PRODUCT DATASHEET

Ready-to-Assay™ 5-HT_{2B} Serotonin Family Receptor Frozen Cells

CATALOG NUMBER: HTS109RTA

Lot: 21C0906

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 DiscoverX 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). The 5-HT_{2B} receptor, which couples to Gq/11 to increase intracellular calcium, is expressed in embryonic and adult cardiovascular tissues, gut and brain from the rat, mouse, and human species. A role for 5-HT_{2B} receptors was suggested in the cardiopathy associated with fenfluramine (Fitzgerald et al., 2000) Eurofins DiscoverX's cloned human 5-HT_{2B} expressing cell line is made in the Chem-8 host, which supports high levels of recombinant 5-HT_{2B} 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_{2B}.

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

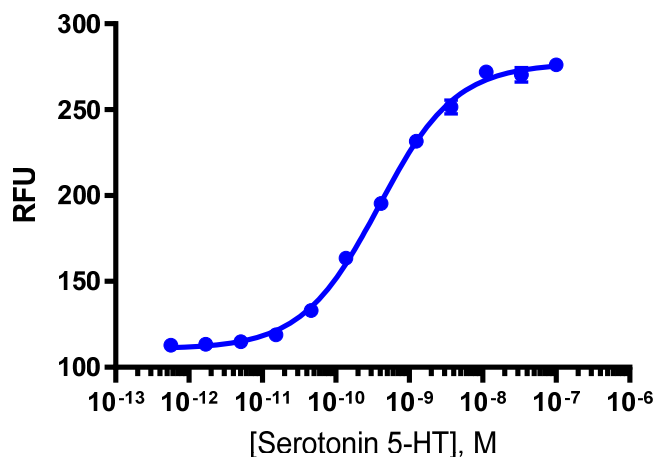


Figure 1. Representative data for activation of 5-HT_{2B} receptor. Calcium flux in 5-HT_{2B}-expressing Chem-8 cell line induced by 5-Hydroxytryptamine (Serotonin). 5-HT_{2B}-expressing Chem-8 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 3-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with EMD camera.

Table 1. Comparison of EC₅₀ values of 5-HT_{2B}-expressing Chem-8 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
5-Hydroxytryptamine	Calcium Flux	0.3	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 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
5-HT 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-8, is a Chinese Hamster Ovarian K-1 cells (CHO K-1) expressing exogenous Gq*s protein.

EXONGENOUS GENE EXPRESSION

HTR2B cDNA (Accession Number: NM_000867; see CODING SEQUENCE below)

CODING SEQUENCE

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                                     ATG GCT CTC TCT TAC AGA GTG TCT GAA
                                     M  A  L  S  Y  R  V  S  E
CTT CAA AGC ACA ATT CCT GAG CAC ATT TTG CAG AGC ACC TTT GTT CAC GTT ATC TCT TCT AAC TGG TCT
L  Q  S  T  I  P  E  H  I  L  Q  S  T  F  V  H  V  I  S  S  N  W  S
GGA TTA CAG ACA GAA TCA ATA CCA GAG GAA ATG AAA CAG ATT GTT GAG GAA CAG GGA AAT AAA CTG CAC
G  L  Q  T  E  S  I  P  E  E  M  K  Q  I  V  E  E  Q  G  N  K  L  H
TGG GCA GCT CTT CTG ATA CTC ATG GTG ATA ATA CCC ACA ATT GGT GGA AAT ACC CTT GTT ATT CTG GCT
W  A  A  L  L  I  L  M  V  I  I  P  T  I  G  G  N  T  L  V  I  L  A
GTT TCA CTG GAG AAG AAG CTG CAG TAT GCT ACT AAT TAC TTT CTA ATG TCC TTG GCG GTG GCT GAT TTG
V  S  L  E  K  K  L  Q  Y  A  T  N  Y  F  L  M  S  L  A  V  A  D  L
CTG GTT GGA TTG TTT GTG ATG CCA ATT GCC CTC TTG ACA ATA ATG TTT GAG GCT ATG TGG CCC CTC CCA
L  V  G  L  F  V  M  P  I  A  L  L  T  I  M  F  E  A  M  W  P  L  P
CTT GTT CTA TGT CCT GCC TGG TTA TTT CTT GAC GTT CTC TTT TCA ACC GCA TCC ATC ATG CAT CTC TGT
L  V  L  C  P  A  W  L  F  L  D  V  L  F  S  T  A  S  I  M  H  L  C
GCC ATT TCA GTG GAT CGT TAC ATA GCC ATC AAA AAG CCA ATC CAG GCC AAT CAA TAT AAC TCA CGG GCT
A  I  S  V  D  R  Y  I  A  I  K  K  P  I  Q  A  N  Q  Y  N  S  R  A
ACA GCA TTC ATC AAG ATT ACA GTG GTG TGG TTA ATT TCA ATA GGC ATT GCC ATT CCA GTC CCT ATT AAA
T  A  F  I  K  I  T  V  V  W  L  I  S  I  G  I  A  I  P  V  P  I  K
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G  I  E  T  D  V  D  N  P  N  N  I  T  C  V  L  T  K  E  R  F  G  D
TTC ATG CTC TTT GGC TCA CTG GCT GCC TTC TTC ACA CCT CTT GCA ATT ATG ATT GTC ACC TAC TTT CTC
F  M  L  F  G  S  L  A  A  F  F  T  P  L  A  I  M  I  V  T  Y  F  L
ACT ATC CAT GCT TTA CAG AAG AAG GCT TAC TTA GTC AAA AAC AAG CCA CCT CAA CGC CTA ACA TGG TTG
T  I  H  A  L  Q  K  K  A  Y  L  V  K  N  K  P  P  Q  R  L  T  W  L
ACT GTG TCT ACA GTT TTC CAA AGG GAT GAA ACA CCT TGC TCG TCA CCG GAA AAG GTG GCA ATG CTG GAT
T  V  S  T  V  F  Q  R  D  E  T  P  C  S  S  P  E  K  V  A  M  L  D
GGT TCT CGA AAG GAC AAG GCT CTG CCC AAC TCA GGT GAT GAA ACA CTT ATG CGA AGA ACA TCC ACA ATT
G  S  R  K  D  K  A  L  P  N  S  G  D  E  T  L  M  R  R  T  S  T  I
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G  K  K  S  V  Q  T  I  S  N  E  Q  R  A  S  K  V  L  G  I  V  F  F
CTC TTT TTG CTT ATG TGG TGT CCC TTC TTT ATT ACA AAT ATA ACT TTA GTT TTA TGT GAT TCC TGT AAC
L  F  L  L  M  W  C  P  F  F  I  T  N  I  T  L  V  L  C  D  S  C  N
CAA ACT ACT CTC CAA ATG CTC CTG GAG ATA TTT GTG TGG ATA GGC TAT GTT TCC TCA GGA GTG AAT CCT
Q  T  T  L  Q  M  L  L  E  I  F  V  W  I  G  Y  V  S  S  G  V  N  P
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L  V  Y  T  L  F  N  K  T  F  R  D  A  F  G  R  Y  I  T  C  N  Y  R
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A  T  K  S  V  K  T  L  R  K  R  S  S  K  I  Y  F  R  N  P  M  A  E
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N  S  K  F  F  K  K  H  G  I  R  N  G  I  N  P  A  M  Y  Q  S  P  M
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R  L  R  S  S  T  I  Q  S  S  S  I  I  L  L  D  T  L  L  L  T  E  N
GAA GGT GAC AAA ACT GAA GAG CGA GTT AGT TAT GTA TGA
E  G  D  K  T  E  E  R  V  S  Y  V  Stp

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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTS109M	ChemiScreen™ 5-HT _{2B} serotonin family receptor membrane prep

REFERENCES

1. Fitzgerald LW *et al.* (2000) Possible role of valvular serotonin 5-HT_{2B} receptors in the cardiopathy associated with fenfluramine. *Mol. Pharmacol.* 57:75-81.
2. Hoyer D *et al.* (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol. Rev.* 46: 157 - 203.

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