

PRODUCT DATASHEET

**Ready-to-Assay™ 5-HT_{2A} Serotonin Family
Receptor Frozen Cells****CATALOG NUMBER: HTS082RTA**

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.

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_{2A}, which couples to Gq/11 to increase intracellular calcium, is widely expressed at central and peripheral sites of 5-HT action, and contributes to many of the physiological effects of 5-HT. The hallucinogenic activity of LSD is mediated in part by its action as a partial to full agonist at 5-HT_{2A}, and the activity of atypical antipsychotics such as clozapine appears to be mediated in part by antagonism of 5-HT_{2A} (Barnes and Sharp, 1999). Cloned human 5-HT_{2A}-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant 5-HT_{2A} 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_{2A}.

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

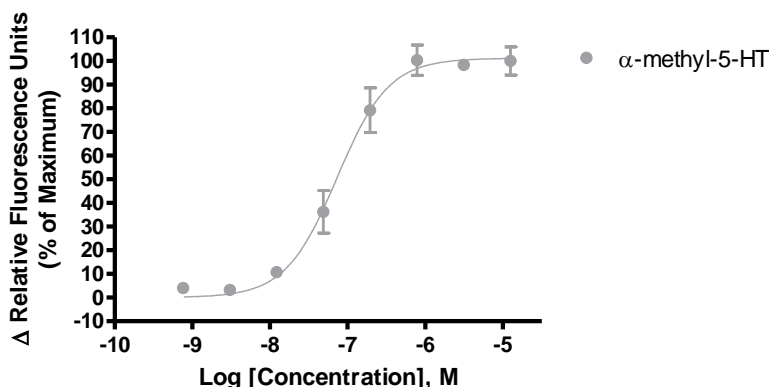


Figure 1. Representative data for activation of 5-HT_{2A} receptor. Calcium flux in 5-HT_{2A}-expressing Chem-1 cell line induced by α -methyl-5-HT. 5-HT_{2A}-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 12,000 RLU (Relative Light Units).

Table 1. EC₅₀ value of 5-HT_{2A}-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
α -methyl-5-HT	Calcium Flux	75	Eurofins Internal Data

ASSAY SETUP

- Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
- 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.
- 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.
- Centrifuge the cell suspension at 190 x g for four minutes
- Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
- Seed cell suspension into appropriate assay microplate (100 μ L/well for 96-well plate, 25 μ L/well for 384-well plate).
- When seeding is complete, place the assay plate at room temperature for 30 minutes.
- Move assay plate to a humidified 37°C 5% CO₂ incubator for 24 hours.
- 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.
- Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca²⁺ dye by dissolving 1mg of Fluo-8 NW in 200 μ L of DMSO. Once dissolved place 10 μ L 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
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

HTR2A cDNA (Accession Number: NM_000621; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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

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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS082M	ChemiScreen™ 5-HT _{2A} 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. 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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