

PRODUCT DATASHEET

ChemiScreen™ 5-HT_{2C} Serotonin Receptor Stable Cell Line

CATALOG NUMBER: HTS132C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N₂.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

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 G_q 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). The 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 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 antagonists of interactions between 5-HT_{2C} and its ligands.

USE RESTRICTIONS

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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 Fluorescence Assay,

APPLICATION DATA

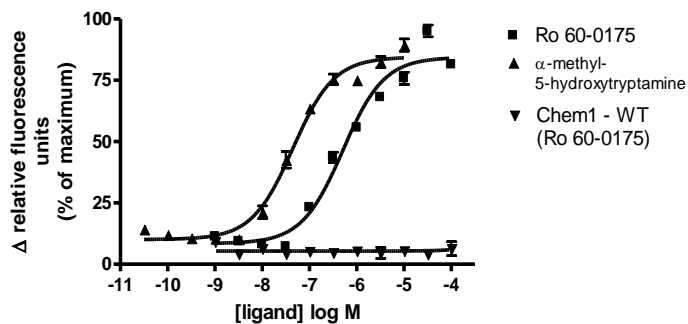


Figure 1. Representative data for activation of 5-HT_{2C} receptor stably expressed in Chem-1 cells induced by α -Methyl-5-hydroxytryptamine using a fluorescent calcium flux assay. 5-HT_{2C}-expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

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

LIGAND	ASSAY	POTENCY EC ₅₀ (nM)	REFERENCE
α-Methyl-5-hydroxytryptamine	Calcium Flux - Fluorescence	45	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC₅₀ and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 μ g/ml	Invivogen: ant-gn-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37°C Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37°C water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37°C, 5% CO₂.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%. At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37° C incubator for additional 2 min.* Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm ²)	Volume (mL)	Total Cell Number (x10 ⁶)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.75	72

ASSAY SETUP

Fluorescence

Table 4. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
α-Methyl-5-hydroxytryptamine	Tocris: 0557
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve 5×10^5 cells/ml (i.e, if collected 5×10^6 TC, $\frac{5 \times 10^6}{5 \times 10^5 / \text{ml}} = 10$ mL volume)
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37°C 5% CO₂ incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human 5-HT_{2C} cDNA (Accession Number: NM_000868; see CODING SEQUENCE below) and promiscuous G protein expressed in a bicistronic vector

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 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 GAA 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-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS132M	ChemiScreen™ 5-HT _{2C} Serotonin 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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