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PRODUCT DATASHEET

ChemiScreen™ 5-HT$_6$ Serotonin Receptor Stable Cell Line

CATALOG NUMBER: HTS111C

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

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-10 host, which supports high levels of functional receptor expression on the cell surface. Chem-10 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

The neurotransmitter serotonin/5-hydroxytryptamine (5-HT) regulates a wide variety of neurological functions. A family of 13 receptors (12 GPCRs and one ion channel) mediate the effects of serotonin. The serotonin receptor 5-HT$_6$ is a G_s coupled receptor expressed solely in the CNS, primarily in the limbic and cortical regions. 5-HT$_6$ appears to play a role in memory and learning, obesity, psychosis, anxiety and epilepsy (Woolley *et al.*, 2004; Fisas *et al.,* 2006). In particular, a 5-HT$_6$-selective agonist caused significant weight loss in a rat model of diet-induced obesity. Cloned human 5-HT$_6$ receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-10 cells with receptor and a promiscuous G protein to couple the receptor to the calcium signaling pathway. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the 5-HT$_6$ receptor.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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 Fluorescence Assay

APPLICATION DATA

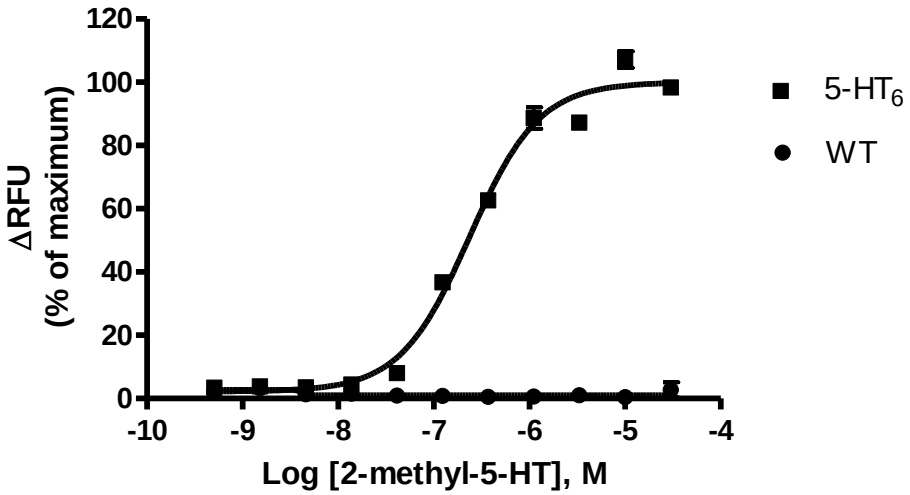

Figure 1. Representative data for activation of the 5-HT_6 receptor stably expressed in Chem-10 cells induced by 2-methyl-5-HT using a fluorescent calcium flux assay. 5-HT_6 –expressing Chem-10 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded 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 6,107 RLU. Similarly parental cells (catalog #: HTSCHEM-10) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of 5-HT_6-expressing Chem-10 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
2-methyl-5-HT	Calcium Flux - Fluorescence	236	Figure 1
2-methyl-5-HT	cAMP accumulation	200	Boess *et al*., 1997

* The cell line was tested and found to have equivalent EC_{50} 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 µg/ml	Invivogen: ant-gn-5
	Hygromycin	250 µg/ml	Invivogen: ant-hg-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 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC 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, 37ºC, 5% CO_2.
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 37º 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	7.0	24
T75	15	2.5	48
T75	15	0.65	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
2-methyl-5-HT	Tocris: 0558-10mg
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 5x10^{5}cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=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 37ºC 5% CO_2 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.

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HOST CELL

Chem-10, an adherent rat hematopoietic cell line expressing endogenous G□15 protein as well as an exogenous proprietary promiscuous Gα protein.

EXOGENOUS GENE EXPRESSION

HTR6 cDNA (Accession Number: NM_000871; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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   1 - ATG GTC CCA GAG CCG GGC CCA ACC GCC AAT AGC ACC CCG GCC TGG GGG GCA GGG CCG CCG  - 60
   1 -  M   V   P   E   P   G   P   T   A   N   S   T   P   A   W   G   A   G   P   P  - 20

  61 - TCG GCC CCG GGG GGC AGC GGC TGG GTG GCG GCC GCG CTG TGC GTG GTC ATC GCG CTG ACG  - 120
  21 -  S   A   P   G   G   S   G   W   V   A   A   A   L   C   V   V   I   A   L   T  - 40

 121 - GCG GCG GCC AAC TCG CTG CTG ATC GCG CTC ATC TGC ACT CAG CCC GCG CTG CGC AAC ACG  - 180
  41 -  A   A   A   N   S   L   L   I   A   L   I   C   T   Q   P   A   L   R   N   T  - 60

 181 - TCC AAC TTC TTC CTG GTG TCG CTC TTC ACG TCT GAC CTG ATG GTG GGG CTG GTG GTG ATG  - 240
  61 -  S   N   F   F   L   V   S   L   F   T   S   D   L   M   V   G   L   V   V   M  - 80

 241 - CCG CCG GCC ATG CTG AAC GCG CTG TAC GGG CGC TGG GTG CTG GCG CGC GGC CTC TGC CTG  - 300
  81 -  P   P   A   M   L   N   A   L   Y   G   R   W   V   L   A   R   G   L   C   L  - 100

 301 - CTC TGG ACC GCC TTC GAC GTG ATG TGC TGC AGC GCC TCC ATC CTC AAC CTC TGC CTC ATC  - 360
 101 -  L   W   T   A   F   D   V   M   C   C   S   A   S   I   L   N   L   C   L   I  - 120

 361 - AGC CTG GAC CGC TAC CTG CTC ATC CTC TCG CCG CTG CGC TAC AAG CTG CGC ATG ACG CCC  - 420
 121 -  S   L   D   R   Y   L   L   I   L   S   P   L   R   Y   K   L   R   M   T   P  - 140

 421 - CTG CGT GCC CTG GCC CTA GTC CTG GGC GCC TGG AGC CTC GCC GCT CTC GCC TCC TTC CTG  - 480
 141 -  L   R   A   L   A   L   V   L   G   A   W   S   L   A   A   L   A   S   F   L  - 160

 481 - CCC CTG CTG CTG GGC TGG CAC GAG CTG GGC CAC GCA CGG CCA CCC GTC CCT GGC CAG TGC  - 540
 161 -  P   L   L   L   G   W   H   E   L   G   H   A   R   P   P   V   P   G   Q   C  - 180

 541 - CGC CTG CTG GTC AGC CTG CCT TTT GTC CTT GTG GCG TCG GGC CTC ACC TTC TTC CTG CCC  - 600
 181 -  R   L   L   V   S   L   P   F   V   L   V   A   S   G   L   T   F   F   L   P  - 200

 601 - TCG GGT GCC ATA TGC TTC ACC TAC TGC AGG ATC CTG CTA GCT GCC CGC AAG CAG GCC GTG  - 660
 201 -  S   G   A   I   C   F   T   Y   C   R   I   L   L   A   A   R   K   Q   A   V  - 220

 661 - CAG GTG GCC TCC CTC ACC ACC GGC ATG GCC AGT CAG GCC TCG GAG ACG CTG CAG GTG CCC  - 720
 221 -  Q   V   A   S   L   T   T   G   M   A   S   Q   A   S   E   T   L   Q   V   P  - 240

 721 - AGG ACC CCA CGC CCA GGG GTG GAG TCT GCT GAC AGC AGG CGT CTA GCC ACG AAG CAC AGC  - 780
 241 -  R   T   P   R   P   G   V   E   S   A   D   S   R   R   L   A   T   K   H   S  - 260

 781 - AGG AAG GCC CTG AAG GCC AGC CTG ACG CTG GGC ATC CTG CTG GGC ATG TTC TTT GTG ACC  - 840
 261 -  R   K   A   L   K   A   S   L   T   L   G   I   L   L   G   M   F   F   V   T  - 280

 841 - TGG TTG CCC TTC TTT GTG GCC AAC ATA GTC CAG GCC GTG TGC GAC TGC ATC TCC CCA GGC  - 900
 281 -  W   L   P   F   F   V   A   N   I   V   Q   A   V   C   D   C   I   S   P   G  - 300

 901 - CTC TTC GAT GTC CTC ACA TGG CTG GGT TAC TGT AAC AGC ACC ATG AAC CCC ATC ATC TAC  - 960
 301 -  L   F   D   V   L   T   W   L   G   Y   C   N   S   T   M   N   P   I   I   Y  - 320

 961 - CCA CTC TTC ATG CGG GAC TTC AAG CGG GCG CTG GGC AGG TTC CTG CCA TGT CCA CGC TGT  - 1020
 321 -  P   L   F   M   R   D   F   K   R   A   L   G   R   F   L   P   C   P   R   C  - 340

1021 - CCC CGG GAG CGC CAG GCC AGC CTG GCC TCG CCA TCA CTG CGC ACC TCT CAC AGC GGC CCC  - 1080
 341 -  P   R   E   R   Q   A   S   L   A   S   P   S   L   R   T   S   H   S   G   P  - 360

1081 - CGG CCC GGC CTT AGC CTA CAG CAG GTG CTG CCG CTG CCC CTG CCG CCG GAC TCA GAT TCG  - 1140
 361 -  R   P   G   L   S   L   Q   Q   V   L   P   L   P   L   P   P   D   S   D   S  - 380

1141 - GAC TCA GAC GCA GGC TCA GGC GGC TCC TCG GGC CTG CGG CTC ACG GCC CAG CTG CTG CTT  - 1200
 381 -  D   S   D   A   G   S   G   G   S   S   G   L   R   L   T   A   Q   L   L   L  - 400

1201 - CCT GGC GAG GCC ACC CAG GAC CCC CCG CTG CCC ACC AGG GCC GCT GCC GCC GTC AAT TTC  - 1260
 401 -  P   G   E   A   T   Q   D   P   P   L   P   T   R   A   A   A   A   V   N   F  - 420

1261 - TTC AAC ATC GAC CCC GCG GAG CCC GAG CTG CGG CCG CAT CCA CTT GGC ATC CCC ACG AAC  - 1320
 421 -  F   N   I   D   P   A   E   P   E   L   R   P   H   P   L   G   I   P   T   N  - 440

1321 - TGA
 441 -  *
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-10	ChemiScreen™ Chem-10 Parental Cell Line (control cells)
HTS111M	ChemiScreen™ 5-HT$_6$Serotonin Receptor Membrane Prep

REFERENCES

1. Boess FG *et al.* (1997) Functional and radioligand binding characterization of rat 5-HT6 receptors stably expressed in HEK293 cells. *Neuropharmacology* 36: 713-720.

2. Fisas A *et al.* (2006) Chronic 5-HT$_6$ receptor modulation by E-6837 induces hypophagia and sustained weight loss in diet-induced obese rats. *Br. J. Pharmacol.* 148: 973-83.

3. Woolley ML *et al.* (2004) 5-HT$_6$ receptors. *Curr. Drug Targets CNS Neurol. Disord.* 3: 59-79.

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