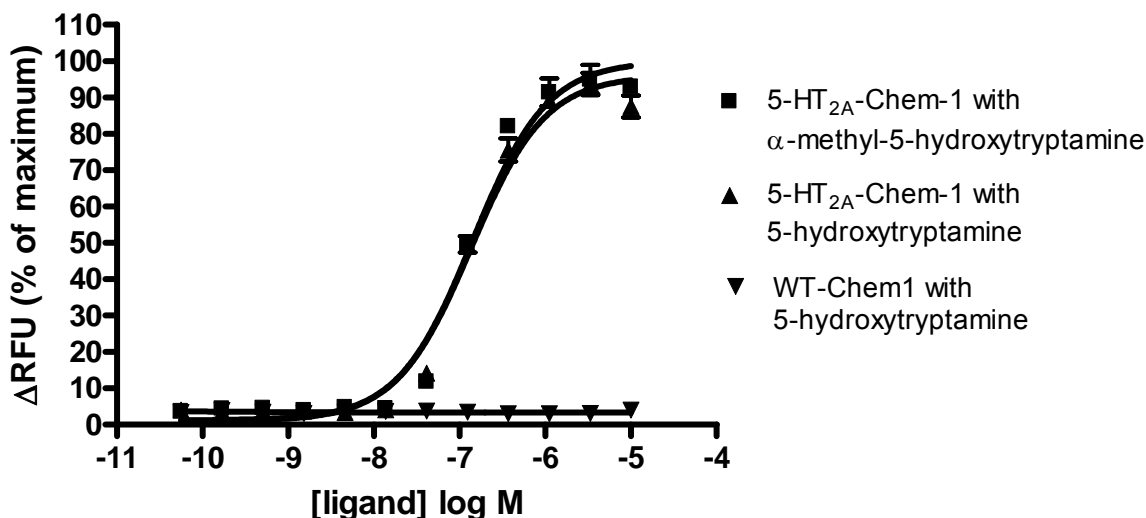


ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT 5-HT<sub>2A</sub> SEROTONIN RECEPTOR

<b>CATALOG NUMBER:</b>	HTS082C	<b>QUANTITY:</b>	2 vials, 1 mL per vial
<b>LOT NUMBER:</b>		<b>CONCENTRATION:</b>	2 x 10 <sup>6</sup> cells/mL

**BACKGROUND:** 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<sub>2A</sub>, which couples to G<sub>q/11</sub> 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<sub>2A</sub>, and the activity of atypical antipsychotics such as clozapine appears to be mediated in part by antagonism of 5-HT<sub>2A</sub> (Barnes and Sharp, 1999). Chemicon's cloned human 5-HT<sub>2A</sub>-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant 5-HT<sub>2A</sub> 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<sub>2A</sub> and its ligands.

**APPLICATIONS:** Calcium flux assay, ligand binding assays



**Figure 1.** Calcium flux in 5-HT<sub>2A</sub>-expressing Chem-1 cell line induced by α-methyl-5-hydroxytryptamine and Serotonin. 5-HT<sub>2A</sub>-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to α-methyl-5-hydroxytryptamine (10<sup>-5</sup> to 10<sup>-10</sup> M) and 5-hydroxytryptamine (10<sup>-5</sup> to 10<sup>-10</sup> M) was determined in triplicate on a Molecular Devices Flex Station.



**Figure 2.** Assay for antagonist activity at 5-HT<sub>2A</sub> by calcium flux assay. 5-HT<sub>2A</sub>-expressing Chem-1 cells were loaded with Fluo-4 and washed. Ketanserin was added to the cells at the final concentration indicated, and incubated for 10 min at 37°C. Calcium flux in response to  $\alpha$ -methyl-5-hydroxytryptamine (280 nM) was determined in duplicate on a Molecular Devices FLIPR<sup>TETRA</sup>™.

SPECIFICATIONS: EC50 for calcium mobilization by  $\alpha$ -methyl-5-hydroxytryptamine: ~ 141 nM  
 EC50 for calcium mobilization by Serotonin: ~ 135 nM  
 IC50 for ketanserin with 2x EC50  $\alpha$ -methyl-5-hydroxytryptamine: 0.74 nM

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, G $\alpha$ 15.

TRANSFECTION: Full-length human HTR2A cDNA encoding 5-HT<sub>2A</sub> (Accession Number: NM\_000621)

**PRESENTATION:**

Cells are frozen at 2 x 10<sup>6</sup> cells/mL in Cell Culture Freezing Media with DMSO (Chemicon catalog number S-002-10F). Cell line tests negative for mycoplasma.

**STORAGE/HANDLING**  
:

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
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. Transfer contents of the vial to a T75 flask containing growth media. Place the flask in a humidified incubator at 37°C with 5% CO<sub>2</sub>.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca<sup>++</sup> and Mg<sup>++</sup> (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37°C with 5% CO<sub>2</sub> until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL 5-HT Chem-1 Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:5 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.

- Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at  $5 \times 10^6$  cells/mL in Chem-1 Freezing Media (cell densities of  $2-10 \times 10^6$  are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at  $-70^\circ\text{C}$  overnight. Store the vials in liquid nitrogen.
- Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays. Cells should be resuspended in 5-HT Chem-1 Plating Media for plating for calcium assay.

**MEDIA:**

## Chem-1 Growth Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020 or equivalent)  
20% heat-inactivated dialyzed FBS (Hyclone SH30079.03)  
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)  
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)  
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)  
G-418 (250ug/mL)

**Note:** *Dialyzed FBS is the recommended serum, as undialyzed FBS contains serotonin that can desensitize 5-HT receptors. Cells proliferate more slowly in media containing dialyzed FBS than in media containing undialyzed FBS. If faster cell growth is desired, the 5-HT<sub>2A</sub>-Chem-1 cells may be propagated in media with undialyzed FBS, but should be returned to media containing dialyzed FBS for at least one passage prior to assay.*

## Chem-1 Plating Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine  
20% heat-inactivated dialyzed FBS  
1x NEAA  
10mM HEPES  
1x Pen-Strep

## Chem-1 Freezing Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine  
20% heat-inactivated FBS (undialyzed FBS is recommended)  
1x NEAA  
10mM HEPES  
1x Pen-Strep  
10% DMSO (cell culture grade)

**REFERENCES:**

Barnes NM and Sharp T (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38, 1083-1152.

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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HUMAN RECOMBINANT 5-HT<sub>2A</sub> SEROTONIN RECEPTOR**

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