

CATALOG NUMBER: HTS110C **QUANTITY:** 2 vials, 1 mL per vial

LOT NUMBER: **CONCENTRATION:** 2 x 10⁶ 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-HT₄ comprises at least 8 isoforms varying at the C-terminus, which are generated by alternative splicing. The expression and distribution of these splice variants differs among organs and tissues with many of them present in several tissues such as atrium, brain, and GI tract (Bockaert *et al.* 2004). To date, all isoforms have been shown to activate adenylyl cyclase *in vitro*, and no difference in signal transduction between C-terminal 5-HT₄ receptor variants has been demonstrated. 5-HT₄ receptors are of potential interest for the treatment of patients with GI motility disorders and Alzheimer's disease (De Maeyer *et al.* 2008; Lezoualc'h 2007). Millipore's cloned human 5-HT_{4B}-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant 5-HT_{4B} expression on the cell surface and contains optimal levels of endogenous promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at 5-HT_{4B}.

APPLICATIONS: Calcium flux assay

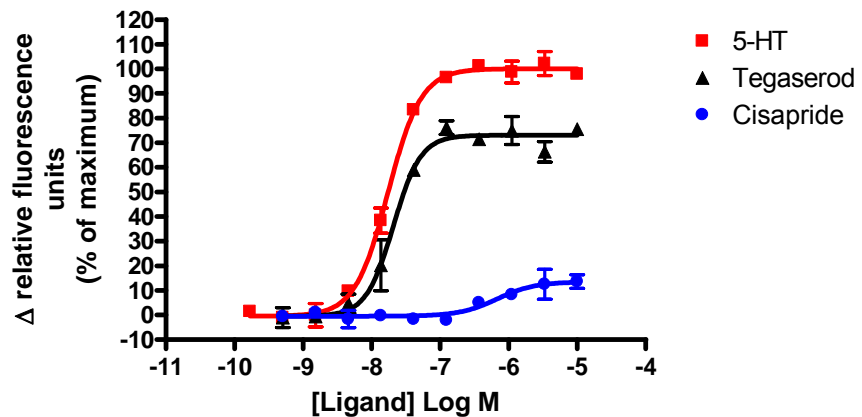


Figure 1. Calcium flux in 5-HT_{4B}-expressing Chem-1 cell line. 5-HT_{4B}-expressing Chem-1 cells were loaded with a no-wash calcium assay kit, and calcium flux in response to 5-HT, cisapride and tegaserod was determined in triplicate on a Molecular Devices FLIPR^{TETRA}. In this experiment, average maximum signal was 2056 RLU. Z' was 0.74 with 5-HT at 4x EC50.

Table I. Comparison of EC50 values of 5-HT_{4B}-expressing Chem-1 cells with values



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described in the literature.

ligand	assay	potency (nM)	Efficacy (% of 5-HT maximum signal)	Reference
5-HT	Calcium	EC50 = 17.5	100%	Figure 1
Tegasero	Calcium	EC50 = 21.0	73%	Figure 1
Cisapride	Calcium	EC50 = 676	13.5%	Figure 1
5-HT	Calcium	EC50 = 12.6	100%	Pindon <i>et al.</i> , 2002
Cisapride	Calcium	n.d.	n.d.	Pindon <i>et al.</i> , 2002
5-HT	cAMP	EC50 = 4.0 nM	100%	Vickery <i>et al.</i> , 2007
Tegasero	cAMP	EC50 = 2.0 nM	127%	Vickery <i>et al.</i> , 2007
Cisapride	cAMP	EC50 = 50 nM	102%	Vickery <i>et al.</i> , 2007

n.d., not detectable

HOST CELLS: Chem-1, a rat adherent cell line of hematopoietic origin expressing the promiscuous G-protein, G_{α15}.

TRANSFECTION: Proprietary plasmid pHS containing HTR4B cDNA encoding 5-HT_{4B} (Accession Number: NM_000870; see CODING SEQUENCE below). The stable clonal cell line was selected by resistance to geneticin, followed by limited dilution cloning. The cell line was tested and found to have equivalent EC50 and signal at 1, 3 and 6 weeks of continuous culture.

PRESENTATION:

Cells are frozen at 2×10^6 cells/mL in 90% fetal bovine serum/10% DMSO. Cell line tests negative for mycoplasma.



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STORAGE/HANDLING:

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. Transfer contents of the vial to a T75 flask containing Growth Media. Place the flask in a humidified incubator at 37°C with 5% CO₂.
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⁺⁺ and Mg⁺⁺ (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% CO₂ 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 Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. 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 x 10⁶ cells/mL in Cryopreservation Media (cell densities of 2-10 x 10⁶ 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°C overnight. Store the vials in liquid nitrogen.
7. 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 Plating Media for plating for calcium assay.

MEDIA:

Growth Media:

UltraCULTURE™ serum-free medium (Lonza 12-725F) supplemented with 2 mM L-glutamine
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
250µg/mL Genetecin/G-418

Plating Media:

UltraCULTURE™ serum-free medium supplemented with 2 mM L-glutamine
1x Nonessential amino acids

Cryopreservation Media:

90% heat-inactivated FBS
10% DMSO (cell culture grade)

EXAMPLE ASSAY CONDITIONS:

1. Cells propagated for screening should be maintained and seeded at less than 90% confluency. Trypsinize cells as above and seed cells in 96-well black-walled, clear bottom plate at 50,000 cells/well in Plating Media. Keep the plate at room temperature for 1 h to allow even cell distribution in the plate, then transfer plate to a humidified incubator at 37°C with 5% CO₂.



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2. Chem-1 derived cell lines have been successfully assayed using multiple commercially-available calcium dye kits following the manufacture's protocols. The protocol described below is a suggested protocol that can be generally applied to most calcium dyes kits.
3. Remove media
4. Wash cells with buffered salt solution
5. Add 100 µL/well calcium dye-loading solution.
6. Incubate the plate for 30 minutes in a humidified incubator at 37°C with 5% CO₂.
7. Incubate the plate for an additional 60 min at 25°C with 5% CO₂.
8. Set-up FLIPR to dispense 50µL/well 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 at 95 uL and dispense rate to 25 µL/sec. Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
9. Ligands are prepared in a white nonbinding surface 96-well plate (Corning 3605).
10. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

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- Vickery RG *et al.* (2007) A comparison of the pharmacological properties of guinea-pig and human recombinant 5-HT₄ receptors. *Br. J. Pharmacol.* 150: 782-791.



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CODING SEQUENCE:

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L T F L S T V I L M A I L G N L L V M V A V 44

TGC TGG GAC AGG CAG CTC AGG AAA ATA AAA ACA AAT TAT TTC ATT GTA TCT CTT GCT TTT GCG GAT 198
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CTG CTG GTT TCG GTG CTG GTG ATG CCC TTT GGT GCC ATT GAG CTG GTT CAA GAC ATC TGG ATT TAT 264
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