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ChemiScreen™ FLASH AEQUORIN CALCIUM-OPTIMIZED STABLE CELL LINE HUMAN RECOMBINANT 5-HT$_{2B}$ SEROTONIN RECEPTOR

CATALOG NUMBER:	HTS109AF	**QUANTITY:**	2 vials, 1 mL per vial
LOT NUMBER:	R0711E0004	**CONCENTRATION:**	2 x 10^6 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). The 5-HT$_{2B}$ receptor, which couples to G$_{q/11}$ to increase intracellular calcium, is expressed in embryonic and adult cardiovascular tissues, gut and brain from the rat, mouse, and human species. A role for 5-HT$_{2B}$ receptors was suggested in the cardiopathy associated with fenfluramine (Fitzgerald *et al.*, 2000). Millipore's cloned human 5-HT$_{2B}$ -expressing cell line is made in the CHO-K1 host which stably expresses a mitochondrially targeted flash mutant form of aequorin. The purified version of this flash variant of aequorin has shown a higher luminescent signal intensity than purfied wildtype aequorin. Thus, the cell line is an ideal tool for screening for antagonists of interactions between 5-HT$_{2B}$ and its ligands.

APPLICATIONS:

Luminescent and fluorescent calcium flux assays, ligand binding assays

(A) **(B)**

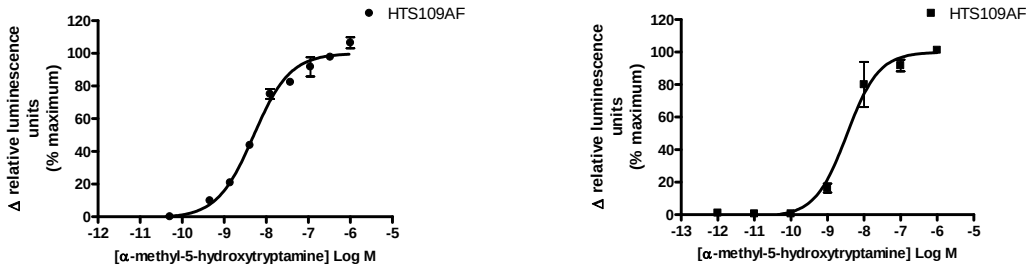

Figure 1. Ligand-induced calcium flux in Flash Aequorin CHO cell line stably transfected with 5-HT$_{2B}$. Flash Aequorin CHO-K1 cell line stably co-expressing 5-HT$_{2B}$ were loaded with 5 μM coelenterazine for 3 h at room temperature. (A) Luminescence in response to α-methyl-5-hydrotryptamine (10^{-6} to $10^{-10.5}$ M) was determined in quadruplicate in a 384 well plate with a FLIPR^{TETRA} with aequorin option (Molecular Devices, now part of MDS Analytical Technologies). Data were collected for area under curve for 70 sec. (B) Luminescence in response to α-methyl-5-hydrotryptamine (10^{-6} to $10^{-10.5}$ M) was determined in duplicate in a 96 well plate with a Perkin Elmer Wallac Victor2. Data were collected for area under curve for 20 sec.

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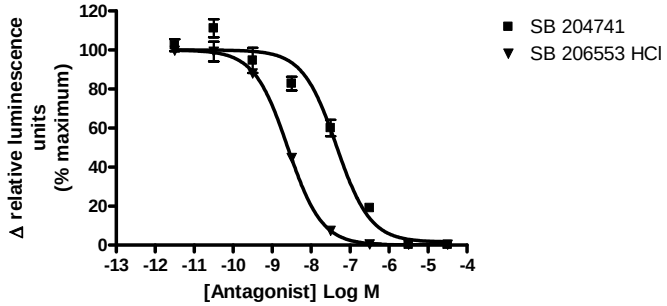

Figure 2. Assay for antagonist activity on ligand-induced calcium flux in Flash Aequorin CHO-K1 cell line stably transfected with 5-HT_{2B}. Flash Aequorin CHO-K1 stably co-expressing 5-HT_{2B} were loaded with 5 μM coelenterazine for 3 h at room temperature. SB 204741 or SB 206553 hydrochloride was added to the cells at the final concentration indicated, and incubated for 10 min at room temperature. Luminescence in response to α-methyl-5-hydrotryptamine (2x EC50 concentration) was determined in duplicate on a Perkin Elmer Wallac Victor2. Data were collected for area under curve for 20 sec.

SPECIFICATIONS: EC50 for calcium mobilization by α-methyl-5-hydrotryptamine:
~ 5.0 nM (FLIPR^{TETRA} system) ~ 3.4 nM (Wallac Victor2)
IC50 for SB 204741: ~ 44.9 nM
for SB 206553 HCl: ~2.5 nM

HOST CELLS: CHO-K1

TRANSFECTION: Full-length human HTR2B cDNA encoding 5-HT_{2B} (Accession Number: X77307) and enhanced flash aequorin

PRESENTATION:

Cells are frozen at 2 x 10^6 cells/mL in 90% heat inactivated dialyzed fetal bovine serum /10% DMSO. 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 37ºC 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 37ºC with 5% CO_2.
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 37ºC with 5% CO_2 until cells begin to round up and detach (2-4 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL CHO Aequorin 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 10^6 cells/mL in Freezing Media (cell densities of 2-10 x 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°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.

MEDIA:

CHO Aequorin Growth Media:
F-12K Nutrient Mixture, Kaighn's Modification with 2 mM L-glutamine
10% heat inactivated dialyzed fetal bovine serum
0.25 mg/ml Geneticin (G418)
0.2 mg/ml Zeocin
100 U/ml each penicillin and streptomycin (from 100x stock, Millipore TMS-AB2-C)

CHO Freezing Media
90% heat inactivated dialyzed fetal bovine serum
10% DMSO

CHO Aequorin Plating Media:
F-12K Nutrient Mixture, Kaighn's Modification with 2 mM L-glutamine
10% heat inactivated dialyzed fetal bovine serum
100 U/ml each penicillin and streptomycin

RECOMMENDED ASSAY CONDITIONS:

1. Seed cells in 96-well white plate (top-read instruments) or opaque-walled, clear bottom plate (bottom-read instruments) overnight at 50,000 cells/well in Chem-1 Aequorin Plating Media.

2. Wash cells once (200 µl/well) with Wash Buffer (HBSS with Ca^{++} and Mg^{++} containing 10 mM HEPES) before loading with 5µM of coelenterazine (Millipore ES016) in wash buffer at room temperature for 3 hours.

   Note: *Luminescence activity has been determined to be optimal at room temperature. Incubation at 37 °C will result in substantially reduced signals.*

3. After loading, wash cells once with Wash Buffer (200 µl/well) prior to addition of ligands.

REFERENCE:

Fitzgerald LW et al. (2000) Possible role of valvular serotonin 5-HT(2B) receptors in the cardiopathy associated with fenfluramine. Mol Pharmacol. 57:75-81.

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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