

PRODUCT DATASHEET

ChemiScreen™ 5-HT_{4B} Serotonin Receptor Stable Cell Line

CATALOG NUMBER: HTS110C

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-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). The 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}.

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.

APPLICATIONS

Calcium Flux Fluorescence Assay

APPLICATION DATA

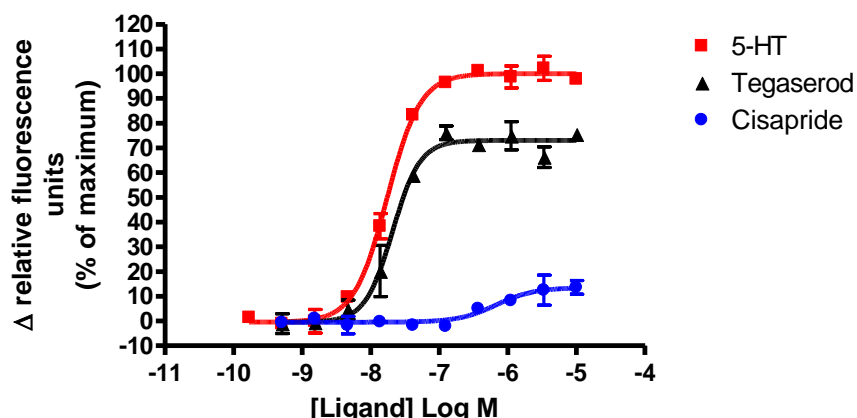


Figure 1. Representative data for activation of the 5-HT_{4B} receptor stably expressed in Chem-1 cells induced by 5-HT, cisapride, and tegaserod using a fluorescent calcium flux assay. 5-HT_{4B}-expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day 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.

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

LIGAND	ASSAY	POTENCY EC ₅₀ (nM)	REFERENCE
5-HT	Calcium Flux - Fluorescence	17.5	Eurofins Internal Data
Tegaserod	Calcium Flux - Fluorescence	21	Eurofins Internal Data
Cisapride	Calcium Flux - Fluorescence	676	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. The Z' value, as defined with response to 5-HT, was 0.74.

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	10mM	Millipore Sigma: H3537
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Gibco:10131-027
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	0.45	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 TM , AM	AAT Bioquest: 21080

5HT	Sigma: H9523-25
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, G α 15.

EXOGENOUS GENE EXPRESSION

HTR4B cDNA encoding 5-HT_{4B} (Accession Number: NM_000870) and promiscuous G protein are expressed in a bicistronic vector

RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS110M	ChemiScreen™ Receptor 5-HT4B Serotonin Receptor Membrane Prep

REFERENCES

1. Bockaert J *et al.* (2004) 5-HT₄ receptors. *Curr. Drug Targets CNS Neurol. Disord.* 3: 39-51.
2. De Maeyer JH *et al.* (2008) 5-HT₄ receptor agonists: similar but not the same. *Neurogastroenterol. Motil.* 20: 99-112.
3. Hoyer D *et al.* 1994 International Union of Pharmacology classification of receptors for 5-HT hydroxytryptamin (Serotonin). *Pharmacol. Rev.* 46: 157-203.
4. Lezoualc'h F (2007) 5-HT₄ receptor and Alzheimer's disease: the amyloid connection. *Exp. Neurol.* 205: 325-329.
5. Pindon A *et al.* (2002) Differences in signal transduction of two 5-HT₄ receptor splice variants: compound specificity and dual coupling with G α s- and G α i/o-proteins. *Mol. Pharmacol.* 61: 85-96.
6. 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.

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

No part of these works may be reproduced in any form without permission in writing.

Limited Use Label License Agreement

In addition to the General Terms & Conditions of Sale for Products and Services section, this Product is subject to Limited Use Label License Agreement. Please go to <https://www.eurofinsdiscoveryservices.com/cms/cms-content/misc/legal-disclaimer/> for more information.

Eurofins Pharma Bioanalytics Services US Inc. is an independent member of Eurofins Discovery Services