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PRODUCT DATASHEET

ChemiScreen™ ET_B Endothelin Receptor Stable Cell Line

CATALOG NUMBER: HTS046C

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

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

This ChemiScreen™ cell line is constructed in the Chem-8 host, which supports high levels of functional receptor expression on the cell surface. Chem-8 cells contain high endogenous levels of promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

The endothelin family of peptides are potent vasoconstrictors synthesized by endothelial cells in both constitutive and inducible pathways. The biological actions of endothelins are mediated by two GPCRs, ET_A and ET_B (Davenport, 2002). Whereas ET_A is the predominant receptor on smooth muscle cells and thus is the primary mediator of the vasoconstrictor activity, ET_B is found on endothelial cells and mediates vasodilation (Nilsson *et al.*, 1997). In addition, ET_B has also been linked to renal failure, congestive heart failure, atherosclerosis, and pulmonary hypertension (D'Orléans-Juste, *et al.,* 2002). A mutation in ET_B has been found to cause Hirschsprung disease-2, a degenerative disease of the digestive tract (Puffenberger *et al*, 1994). Cloned human ET_B receptor-expressing ChemiScreen cells were constructed by stable transfection of CHO cells with ET_B. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the ET_B 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

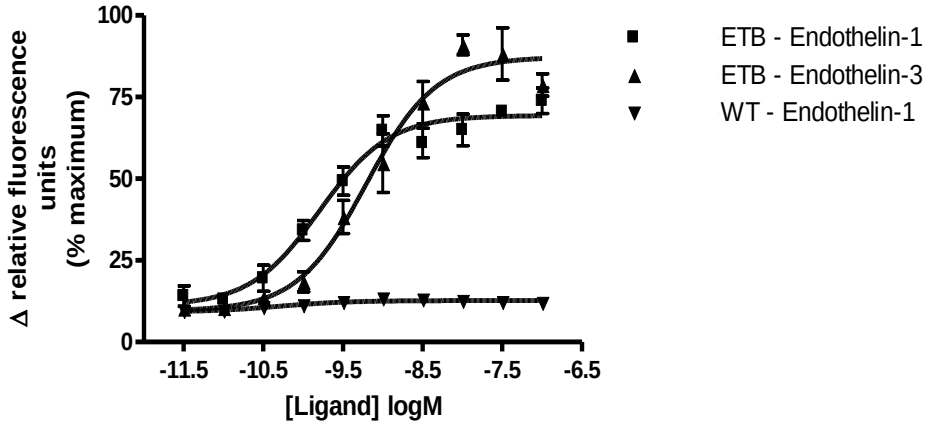

Figure 1. Representative data for activation of ET_B receptor stably expressed in Chem-8 cells induced by ligands using a fluorescent calcium flux assay. ET_B–expressing Chem-8 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 8,000 RLU. Similarly parental cells were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of ET_B-expressing Chem-8 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Endothelin-1	Calcium Flux - Fluorescence	0.2	Eurofins Internal Data
Endothelin-3	Calcium Flux - Fluorescence	0.6	Eurofins Internal Data

* 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	F-12 Kaighn's Modification	-	Hyclone: SH30526.01
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-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	4.0	24
T75	15	1.5	48
T75	15	0.35	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
Endothelin-1 ligand	Tocris: 1160
Endothelin-3 ligand	Tocris: 1162
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.

HOST CELL

Chem-8

EXOGENOUS GENE EXPRESSION

EDNRB cDNA (Accession Number: NM_000115; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                        ATG CAG CCG CCT CCA AGT CTG
                                                         M   Q   P   R   P   S   L

TGC GGA CGC GCC CTG GTT GCG CTG GTT CTT GCC TGC GGC CTG TCG CGG ATC TGG GGA GAG
 C   G   R   A   L   V   A   L   V   L   A   C   G   L   S   R   I   W   G   E

GAG AGA GGC TTC CCG CCT GAC AGG GCC ACT CCG CTT TTG CAA ACC GCA GAG ATA ATG ACG
 E   R   G   F   P   P   D   R   A   T   P   L   L   Q   T   A   E   I   M   T

CCA CCC ACT AAG ACC TTA TGG CCC AAG GGT TCC AAC GCC AGT CTG GCG CGG TCG TTG GCA
 P   P   T   K   T   L   W   P   K   G   S   N   A   S   L   A   R   S   L   A

CCT GCG GAG GTG CCT AAA GGA GAC AGG ACG GCA GGA TCT CCG CCA CGC ACC ATC TCC CCT
 P   A   E   V   P   K   G   D   R   T   A   G   S   P   P   R   T   I   S   P

CCC CCG TGC CAA GGA CCC ATC GAG ATC AAG GAG ACT TTC AAA TAC ATC AAC ACG GTT GTG
 P   P   C   Q   G   P   I   E   I   K   E   T   F   K   Y   I   N   T   V   V

TCC TGC CTT GTG TTC GTG CTG GGG ATC ATC GGG AAC TCC ACA CTT CTG AGA ATT ATC TAC
 S   C   L   V   F   V   L   G   I   I   G   N   S   T   L   L   R   I   I   Y

AAG AAC AAG TGC ATG CGA AAC GGT CCC AAT ATC TTG ATC GCC AGC TTG GCT CTG GGA GAC
 K   N   K   C   M   R   N   G   P   N   I   L   I   A   S   L   A   L   G   D

CTG CTG CAC ATC GTC ATT GAC ATC CCT ATC AAT GTC TAC AAG CTG CTG GCA GAG GAC TGG
 L   L   H   I   V   I   D   I   P   I   N   V   Y   K   L   L   A   E   D   W

CCA TTT GGA GCT GAG ATG TGT AAG CTG GTG CCT TTC ATA CAG AAA GCC TCC GTG GGA ATC
 P   F   G   A   E   M   C   K   L   V   P   F   I   Q   K   A   S   V   G   I

ACT GTG CTG AGT CTA TGT GCT CTG AGT ATT GAC AGA TAT CGA GCT GTT GCT TCT TGG AGT
 T   V   L   S   L   C   A   L   S   I   D   R   Y   R   A   V   A   S   W   S

AGA ATT AAA GGA ATT GGG GTT CCA AAA TGG ACA GCA GTA GAA ATT GTT TTG ATT TGG GTG
 R   I   K   G   I   G   V   P   K   W   T   A   V   E   I   V   L   I   W   V

GTC TCT GTG GTT CTG GCT GTC CCT GAA GCC ATA GGT TTT GAT ATA ATT ACG ATG GAC TAC
 V   S   V   V   L   A   V   P   E   A   I   G   F   D   I   I   T   M   D   Y

AAA GGA AGT TAT CTG CGA ATC TGC TTG CTT CAT CCC GTT CAG AAG ACA GCT TTC ATG CAG
 K   G   S   Y   L   R   I   C   L   L   H   P   V   Q   K   T   A   F   M   Q

TTT TAC AAG ACA GCA AAA GAT TGG TGG CTG TTC AGT TTC TAT TTC TGC TTG CCA TTG GCC
 F   Y   K   T   A   K   D   W   W   L   F   S   F   Y   F   C   L   P   L   A

ATC ACT GCA TTT TTT TAT ACA CTA ATG ACC TGT GAA ATG TTG AGA AAG AAA AGT GGC ATG
 I   T   A   F   F   Y   T   L   M   T   C   E   M   L   R   K   K   S   G   M

CAG ATT GCT TTA AAT GAT CAC CTA AAG CAG AGA CGG GAA GTG GCC AAA ACC GTC TTT TGC
 Q   I   A   L   N   D   H   L   K   Q   R   R   E   V   A   K   T   V   F   C

CTG GTC CTT GTC TTT GCC CTC TGC TGG CTT CCC CTT CAC CTC AGC AGG ATT CTG AAG CTC
 L   V   L   V   F   A   L   C   W   L   P   L   H   L   S   R   I   L   K   L

ACT CTT TAT AAT CAG AAT GAT CCC AAT AGA TGT GAA CTT TTG AGC TTT CTG TTG GTA TTG
 T   L   Y   N   Q   N   D   P   N   R   C   E   L   L   S   F   L   L   V   L

GAC TAT ATT GGT ATC AAC ATG GCT TCA CTG AAT TCC TGC ATT AAC CCA ATT GCT CTG TAT
 D   Y   I   G   I   N   M   A   S   L   N   S   C   I   N   P   I   A   L   Y

TTG GTG AGC AAA AGA TTC AAA AAC TGC TTT AAG TCA TGC TTA TGC TGC TGG TGC CAG TCA
 L   V   S   K   R   F   K   N   C   F   K   S   C   L   C   C   W   C   Q   S

TTT GAA GAA AAA CAG TCC TTG GAG GAA AAG CAG TCG TGC TTA AAG TTC AAA GCT AAT GAT
 F   E   E   K   Q   S   L   E   E   K   Q   S   C   L   K   F   K   A   N   D

CAC GGA TAT GAC AAC TTC CGT TCC AGT AAT AAA TAC AGC TCA TCT TGA
 H   G   Y   D   N   F   R   S   S   N   K   Y   S   S   S  Stp
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RELATED PRODUCTS

Product Number	Description
HTS046M	ChemiScreen™ ET_B Endothelin receptor membrane prep

REFERENCES

1. Davenport AP (2002) International Union of Pharmacology. XXIX. Update on Endothelin Receptor Nomenclature. *Pharmacol. Rev.* 54: 219-226.
2. Nilsson T, *et al.* (1997) Presence of contractile endothelin-A and dilatory endothelin-B receptors in human cerebral arteries. *Neurosurgery.* 40: 346–351;comment 351–353
3. D'Orléans-Juste P, *et al* (2002) Function of the $endothelin_B$ receptor in cardiovascular physiology and pathophysiology. *Pharmacol Ther.* 95: 221-238.
4. Puffenberger EG, *et al* (1994) A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. *Cell* 79: 1257-1266.

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