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PRODUCT DATASHEET

ChemiScreen™ β_2 Adrenergic Receptor Stable Cell Line

CATALOG NUMBER: HTS073C

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

STORAGE: Vials are to be stored in liquid N_2.

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.

The endogenous catecholamines epinephrine, and norepinephrine have profound effects on smooth muscle activity, cardiac function, carbohydrate and fat metabolism, hormone secretion, neurotransmitter release, and central nervous system actions. These activities are mediated by GPCRs belonging to two subfamilies, the α- and β-adrenergic receptors (Bylund *et al*., 1994). The β-adrenergic receptors, primarily the β_2 subtype, mediate relaxation of smooth muscle in many tissues, and β_2-selective agonists are the preferred drugs for stimulating bronchodilation in the treatment of asthma and chronic obstructive pulmonary disease (Sears and Lotvall, 2005). Activation of the β-adrenergic receptors, primarily the β1 subtype and to a lesser extent the β2 subtype, acutely increases heart rate, cardiac output, and cardiac automaticity, and chronically increases cardiac myocyte apoptosis. As a result, β -adrenergic receptor antagonists (β blockers) are effective in the treatment of congestive heart failure and arrhythmia (Feldman *et al*., 2005). Cloned human β_2 receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-1 cells with β_2 and a promiscuous G protein to couple the receptor to the calcium signaling pathway. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the β_2 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

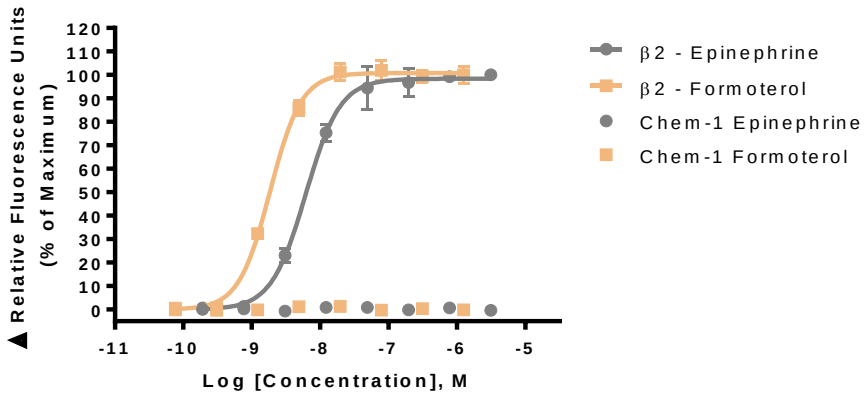

Figure 1. Representative data for activation of β_2 receptor stably expressed in Chem-1 cells induced by Epinephrine and Formoterol using a fluorescent calcium flux assay. β_2–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded 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. Maximal fluorescence signal obtained in this experiment was 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of β_2-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Epinephrine	Calcium Flux - Fluorescence	6	Eurofins Internal Data
Formoterol	Calcium Flux - Fluorescence	2	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. The Z' value, as defined with response to 10µM Epinephrine, was 0.85.

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	1X	Hyclone: SH30237.01
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	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™, AM	AAT Bioquest: 21080
Epinephrine ligand	Sigma: E1635
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-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

β_2 cDNA (Accession Number: NM_000024; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG GGG CAA CCC GGG AAC GGC AGC GCC TTC TTG
                                                 M   G   Q   P   G   N   G   S   A   F   L

CTG GCA CCC AAT AGA AGC CAT GCG CCG GAC CAC GAC GTC ACG CAG CAA AGG GAC GAG GTG TGG GTG GTG
 L   A   P   N   G   S   H   A   P   D   H   D   V   T   Q   Q   R   D   E   V   W   V   V

GGC ATG GGC ATC GTC ATG TCT CTC ATC GTC CTG GCC ATC GTG TTT GGC AAT GTG CTG GTC ATC ACA GCC
 G   M   G   I   V   M   S   L   I   V   L   A   I   V   F   G   N   V   L   V   I   T   A

ATT GCC AAG TTC GAG CGT CTG CAG ACG GTC ACC AAC TAC TTC ATC ACT TCA CTG GCC TGT GCT GAT CTG
 I   A   K   F   E   R   L   Q   T   V   T   N   Y   F   I   T   S   L   A   C   A   D   L

GTC ATG GGC CTG GCA GTG GTG CCC TTT GGG GCC GCC CAT ATT CTT ATG AAA ATG TGG ACT TTT GGC AAC
 V   M   G   L   A   V   V   P   F   G   A   A   H   I   L   M   K   M   W   T   F   G   N

TTC TGG TGC GAG TTT TGG ACT TCC ATT GAT GTG CTG TGC GTC ACG GCC AGC ATT GAG ACC CTG TGC GTG
 F   W   C   E   F   W   T   S   I   D   V   L   C   V   T   A   S   I   E   T   L   C   V

ATC GCA GTG GAT CGC TAC TTT GCC ATT ACT TCA CCT TTC AAG TAC CAG AGC CTG CTG ACC AAG AAT AAG
 I   A   V   D   R   Y   F   A   I   T   S   P   F   K   Y   Q   S   L   L   T   K   N   K

GCC CGG GTG ATC ATT CTG ATG GTG TGG ATT GTG TCA GGC CTT ACC TCC TTC TTG CCC ATT CAG ATG CAC
 A   R   V   I   I   L   M   V   W   I   V   S   G   L   T   S   F   L   P   I   Q   M   H

TGG TAC CGG GCC ACC CAC CAG GAA GCC ATC AAC TGC TAT GCC AAT GAG ACC TGC TGT GAC TTC TTC ACG
 W   Y  R→R  A   T   H   Q   E   A   I   N   C   Y   A   N   E   T   C   C   D   F   F   T

AAC CAA GCC TAT GCC ATT GCC TCT TCC ATC GTG TCC TTC TAC GTT CCC CTG GTG ATC ATG GTC TTC GTC
 N   Q   A   Y   A   I   A   S   S   I   V   S   F   Y   V   P   L   V   I   M   V   F   V

TAC TCC AGG GTC TTT CAG GAG GCC AAA AGG CAG CTC CAG AAG ATT GAC AAA TCT GAG GGC CGC TTC CAT
 Y   S   R   V   F   Q   E   A   K   R   Q   L   Q   K   I   D   K   S   E   G   R   F   H

GTC CAG AAC CTT AGC CAG GTG GAG CAG GAT GGG CGG ACG GGG CAT GGA CTC CGC AGA TCT TCC AAG TTC
 V   Q   N   L   S   Q   V   E   Q   D   G   R   T   G   H   G   L   R   R   S   S   K   F

TGC TTG AAG GAG CAC AAA GCC CTC AAG ACG TTA GGC ATC ATC ATG GGC ACT TTC ACC CTC TGC TGG CTG
 C   L   K   E   H   K   A   L   K   T   L   G   I   I   M   G   T   F   T   L   C   W   L

CCC TTC TTC ATC GTT AAC ATT GTG CAT GTG ATC CAG GAT AAC CTC ATC CGT AAG GAA GTT TAC ATC CTC
 P   F   F   I   V   N   I   V   H   V   I   Q   D   N   L   I   R   K   E   V   Y   I   L

CTA AAT TGG ATA GGC TAT GTC AAT TCT GGT TTC AAT CCC CTT ATC TAC TGC CGG AGC CCA GAT TTC AGG
 L   N   W   I   G   Y   V   N   S   G   F   N   P   L   I   Y   C   R   S   P   D   F   R

ATT GCC TTC CAG GAG CTT CTG TGC CTG CGC AGG TCT TCT TTG AAG GCC TAT GGG AAT GGC TAC TCC AGC
 I   A   F   Q   E   L   L   C   L   R   R   S   S   L   K   A   Y   G   N   G   Y   S   S

AAC GGC AAC ACA GGG GAG CAG AGT GGA TAT CAC GTG GAA CAG GAG AAA GAA AAT AAA CTG CTG TGT GAA
 N   G   N   T   G   E   Q   S   G   Y   H   V   E   Q   E   K   E   N   K   L   L   C   E

GAC CTC CCA GGC ACG GAA GAC TTT GTG GGC CAT CAA GGT ACT GTG CCT AGC GAT AAC ATT GAT TCA CAA
 D   L   P   G   T   E   D   F   V   G   H   Q   G   T   V   P   S   D   N   I   D   S   Q

GGG AGG AAT TGT AGT ACA AAT GAC TCA CTG CTG TGA
 G   R   N   C   S   T   N   D   S   L   L  Stp
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS073M	ChemiScreen™ β_2 Adrenergic receptor membrane prep

REFERENCES

1. Bylund DB et al. (1994). IV. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.* 46: 121-136.
2. Feldman DS et al. (2005) Mechanisms of Disease: β-adrenergic receptors—alterations in signal transduction and pharmacogenomics in heart failure. *Nat. Clin. Pract. Cardiovasc. Med*. 2: 475-83.
3. Sears MR and Lotvall J (2005) Past, present and future—β_2-adrenoceptor agonists in asthma management. *Respir. Med.* 99: 152-70.

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