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PRODUCT DATASHEET

ChemiScreen™ β_1 Adrenergic Receptor Stable Cell Line

CATALOG NUMBER: HTS104C

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 three members of the β-adrenergic receptor family, β_1, β_2 and β_3, couple to G_s to increase cAMP upon activation. In the heart, the β_1 receptor constitutes 70-80% of the β-adrenergic receptors. Activation of cardiac β-adrenergic receptors, acutely increases heart rate, cardiac output, and cardiac automaticity, and chronically increases cardiac myocyte apoptosis. In failing hearts, the β_1 subtype is downregulated and desenstitized, probably as a result of increased catecholamine levels. As a result, β-adrenergic receptor antagonists (β blockers) are effective in the treatment of congestive heart failure and arrhythmia (Lohse *et al.*, 2003). Cloned human β_1 receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-1 cells with β_1 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_1 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, cAMP accumulation

APPLICATION DATA

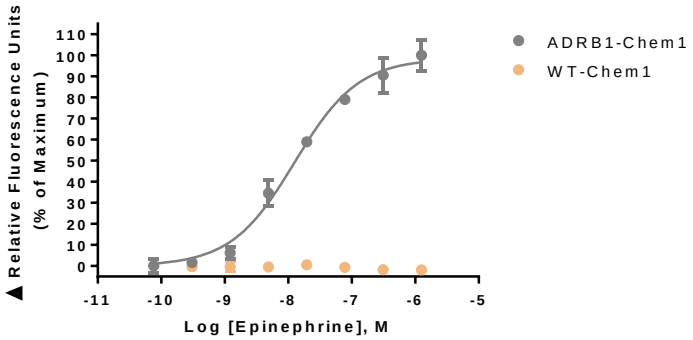

Figure 1. Representative data for activation of β_1 receptor stably expressed in Chem-1 cells induced by epinephrine using a fluorescent calcium flux assay. β_1–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 24,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

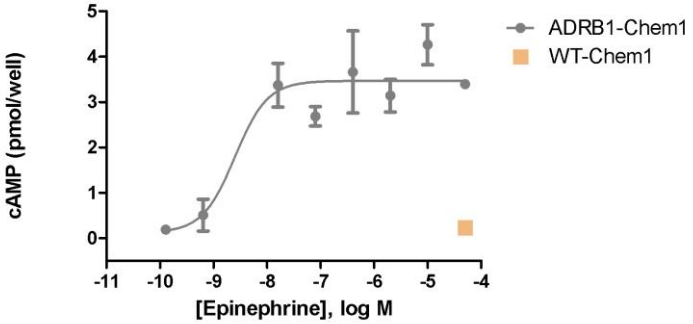

Figure 2. Representative data for activation of β_1 receptor stably expressed in Chem-1 cells induced by epinephrine using a cAMP accumulation assay. β_1–expressing Chem-1 cells were seeded at 100,000 cells per well into a 96-well plate, and the following day the cells were treated with epinephrine for 15 minutes in the presence of 2.0 mM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal cAMP response obtained in this experiment was 4.5pmol/well. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of β_1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Epinephrine	Calcium Flux - Fluorescence	13	Eurofins Internal Data
Epinephrine	cAMP accumulation	2.5	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 1µM epinephrine, was 0.7.

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^{TM}, 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.

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HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human ADRβ1 cDNA (Accession Number: NM_000684; see CODING SEQUENCE below) expressed from a proprietary pBill plasmid.

CODING SEQUENCE

```
ATG GGC GCG GGG GTG CTT GTC CTG GGC GCC TCC GAG CCC GGT AAC CTG TCG TCG GCC GCA CCG CTC CCC
 M   G   A   G   V   L   V   L   G   A   S   E   P   G   N   L   S   S   A   A   P   L   P

GAC GGC GCG GCC ACC GCG GCG CGG CTG CTG GTG CCC GCG TCG CCG CCC GCC TCG TTG CTG CCT CCC GCC
 D   G   A   A   T   A   A   R   L   L   V   P   A   S   P   P   A   S   L   L   P   P   A

AGC GAA AGC CCC GAG CCG CTG TCT CAG CAG TGG ACA GCG GGC ATG GGT CTG CTG ATG GCG CTC ATC GTG
 S   E   S   P   E   P   L   S   Q   Q   W   T   A   G   M   G   L   L   M   A   L   I   V

CTG CTC ATC GTG GCG GGC AAT GTG CTG GTG ATC GTG GCC ATC GCC AAG ACG CCG CGG CTG CAG ACG CTC
 L   L   I   V   A   G   N   V   L   V   I   V   A   I   A   K   T   P   R   L   Q   T   L

ACC AAC CTC TTC ATC ATG TCC CTG GCC AGC GCC GAC CTG GTC ATG GGG CTG CTG GTG GTG CCG TTC GGG
 T   N   L   F   I   M   S   L   A   S   A   D   L   V   M   G   L   L   V   V   P   F   G

GCC ACC ATC GTG GTG TGG GGC CGC TGG GAG TAC GGC TCC TTC TTC TGC GAG CTG TGG ACC TCA GTG GAC
 A   T   I   V   V   W   G   R   W   E   Y   G   S   F   F   C   E   L   W   T   S   V   D

GTG CTG TGC GTG ACG GCC AGC ATC GAG ACC CTG TGT GTC ATT GCC CTG GAC CGC TAC CTC GCC ATC ACC
 V   L   C   V   T   A   S   I   E   T   L   C   V   I   A   L   D   R   Y   L   A   I   T

TCG CCC TTC CGC TAC CAG AGC CTG CTG ACG CGC GCG CGG GCG CGG GGC CTC GTG TGC ACC GTG TGG GCC
 S   P   F   R   Y   Q   S   L   L   T   R   A   R   A   R   G   L   V   C   T   V   W   A

ATC TCG GCC CTG GTG TCC TTC CTG CCC ATC CTC ATG CAC TGG TGG CGG GCG GAG AGC GAC GAG GCG CGC
 I   S   A   L   V   S   F   L   P   I   L   M   H   W   W   R   A   E   S   D   E   A   R

CGC TGC TAC AAC GAC CCC AAG TGC TGC GAC TTC GTC ACC AAC CGG GCC TAC GCC ATC GCC TCG TCC GTA
 R   C   Y   N   D   P   K   C   C   D   F   V   T   N   R   A   Y   A   I   A   S   S   V

GTC TCC TTC TAC GTG CCC CTG TGC ATC ATG GCC TTC GTG TAC CTG CGG GTG TTC CGC GAG GCC CAG AAG
 V   S   F   Y   V   P   L   C   I   M   A   F   V   Y   L   R   V   F   R   E   A   Q   K

CAG GTG AAG AAG ATC GAC AGC TGC GAG CGC CGT TTC CTC GGC GGC CCA GCG CGG CCG CCC TCG CCC TCG
 Q   V   K   K   I   D   S   C   E   R   R   F   L   G   G   P   A   R   P   P   S   P   S

CCC TCG CCC GTC CCC GCG CCC GCG CCG CCG CCC GGA CCC CCG CGC CCC GCC GCC GCC GCC GCC ACC GCC
 P   S   P   V   P   A   P   A   P   P   P   G   P   P   R   P   A   A   A   A   A   T   A

CCG CTG GCC AAC GGG CGT GCG GGT AAG CGG CGG CCC TCG CGC CTC GTG GCC CTG CGC GAG CAG AAG GCG
 P   L   A   N   G   R   A   G   K   R   R   P   S   R   L   V   A   L   R   E   Q   K   A

CTC AAG ACG CTG GGC ATC ATC ATG GGC GTC TTC ACG CTC TGC TGG CTG CCC TTC TTC CTG GCC AAC GTG
 L   K   T   L   G   I   I   M   G   V   F   T   L   C   W   L   P   F   F   L   A   N   V

GTG AAG GCC TTC CAC CGC GAG CTG GTG CCC GAC CGC CTC TTC GTC TTC TTC AAC TGG CTG GGC TAC GCC
 V   K   A   F   H   R   E   L   V   P   D   R   L   F   V   F   F   N   W   L   G   Y   A

AAC TCG GCC TTC AAC CCC ATC ATC TAC TGC CGC AGC CCC GAC TTC CGC AAG GCC TTC CAG GGA CTG CTC
 N   S   A   F   N   P   I   I   Y   C   R   S   P   D   F   R   K   A   F   Q   G   L   L

TGC TGC GCG CGC AGG GCT GCC CGC CGG CGC CAC GCG ACC CAC GGA GAC CGG CCG CGC GCC TCG GGC TGT
 C   C   A   R   R   A   A   R   R   R   H   A   T   H   G   D   R   P   R   A   S   G   C

CTG GCC CGG CCC GGA CCC CCG CCA TCG CCC GGG GCC GCC TCG GAC GAC GAC GAC GAC GAT GTC GTC GGG
 L   A   R   P   G   P   P   P   S   P   G   A   A   S   D   D   D   D   D   D   V   V   G

GCC ACG CCG CCC GCG CGC CTG CTG GAG CCC TGG GCC GGC TGC AAC GGC GGG GCG GCG GCG GAC AGC GAC
 A   T   P   P   A   R   L   L   E   P   W   A   G   C   N   G   G   A   A   A   D   S   D

TCG AGC CTG GAC GAG CCG TGC CGC CCC GGC TTC GCC TCG GGA TCC AAG GTG TGA
 S   S   L   D   E   P   C   R   P   G   F   A   S   G   S   K   V  Stp
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS104M	ChemiScreen™ β_1 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. Lohse MJ *et al.* (2003) What is the role of β-adrenergic signaling in heart failure? *Circ. Res.* 93: 896-906.

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