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PRODUCT DATASHEET

ChemiScreen™ Alpha1B Adrenergic Receptor Stable Cell Line

CATALOG NUMBER: HTS158C

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-adrenoceptors (Bylund et al., 1994). The three members of the α1 subclass of adrenoceptors, α1A, α1B and α 1D, couple to Gq, and promote contraction of vascular and urinary tract smooth muscle, relaxation of intestinal smooth muscle, increased contractile force in the heart, and glycogenolysis and gluconeogenesis in the liver. The different subtypes have overlapping distributions and variably contribute to these effects depending on species and tissue. Overexpression of a constitutively active α1b mutant in the heart of transgenic mice resulted in cardiac hypertrophy with increased heart weight/body weight ratios. Analysis of α1B knock out mice has provided evidence that α1B is a mediator of blood pressure and aortic contractile responses induced by α1B agonists (Milano et al., 1994). The locomotor and rewarding effects of pysochostimulants and opiates were suppressed in mice lacking α1B-adrenergic receptors (Drouin et al. 2002). Our cloned human α1B-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant α1B expression on the cell surface and contains high levels of the promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between α1B and its ligands.

USE RESTRICTIONS

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WARNINGS

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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

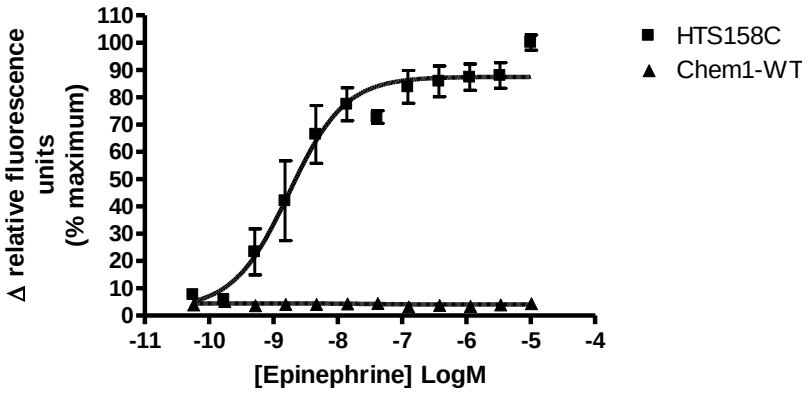

Figure 1. Calcium flux in α1B–expressing Chem-1 cell line induced by Epinephrine. α1B expressing Chem-1 cells and Wild-Type Chem-1 cells were loaded with Fluo-4 NW and calcium flux in response to Epinephrine ($10^{-5.0}$ to $10^{-10.25}$M) was determined in triplicate on a Molecular Devices FLIPR TETRA.

Table 1. EC_{50} value of α1B–expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Epinephrine	Calcium Flux - Fluorescence	1.7	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	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	EMD Millipore: TMS-003-C
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: 1635
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.
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

Full-length human ADRA1B cDNA encoding α_{1B} (Accession Number: NM_000679.3)

CODING SEQUENCE

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ATG AAT CCC GAC CTG GAC ACC GGC CAC AAC ACA TCA GCA CCT GCC CAC TGG GGA GAG TTG AAA AAT GCC AAC TTC ACT
 M   N   P   D   L   D   T   G   H   N   T   S   A   P   A   H   W   G   E   L   K   N   A   N   F   T

GGC CCC AAC CAG ACC TCG AGC AAC TCC ACA CTG CCC CAG CTG GAC ATC ACC AGG GCC ATC TCT GTG GGC CTG GTG CTG
 G   P   N   Q   T   S   S   N   S   T   L   P   Q   L   D   I   T   R   A   I   S   V   G   L   V   L

GGC GCC TTC ATC CTC TTT GCC ATC GTG GGC AAC ATC CTA GTC ATC TTG TCT GTG GCC TGC AAC CGG CAC CTG CGG ACG
 G   A   F   I   L   F   A   I   V   G   N   I   L   V   I   L   S   V   A   C   N   R   H   L   R   T

CCC ACC AAC TAC TTC ATT GTC AAC CTG GCC ATG GCC GAC CTG CTG TTG AGC TTC ACC GTC CTG CCC TTC TCA GCG GCC
 P   T   N   Y   F   I   V   N   L   A   M   A   D   L   L   L   S   F   T   V   L   P   F   S   A   A

CTA GAG GTG CTC GGC TAC TGG GTG CTG GGG CGG ATC TTC TGT GAC ATC TGG GCA GCC GTG GAT GTC CTG TGC TGC ACA
 L   E   V   L   G   Y   W   V   L   G   R   I   F   C   D   I   W   A   A   V   D   V   L   C   C   T

GCG TCC ATT CTG AGC CTG TGC GCC ATC TCC ATC GAT CGC TAC ATC GGG GTG CGC TAC TCT CTG CAG TAT CCC ACG CTG
 A   S   I   L   S   L   C   A   I   S   I   D   R   Y   I   G   V   R   Y   S   L   Q   Y   P   T   L
```

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GTC ACC CGG AGG AAG GCC ATC TTG GCG CTG CTC AGT GTC TGG GTC TTG TCC ACC GTC ATC TCC ATC GGG CCT CTC CTT
 V   T   R   R   K   A   I   L   A   L   L   S   V   W   V   L   S   T   V   I   S   I   G   P   L   L

GGG TGG AAG GAG CCG GCA CCC AAC GAT GAC AAG GAG TGC GGG GTC ACC GAA GAA CCC TTC TAT GCC CTC TTC TCC TCT
 G   W   K   E   P   A   P   N   D   D   K   E   C   G   V   T   E   E   P   F   Y   A   L   F   S   S

CTG GGC TCC TTC TAC ATC CCT CTG GCG GTC ATT CTA GTC ATG TAC TGC CGT GTC TAT ATA GTG GCC AAG AGA ACC ACC
 L   G   S   F   Y   I   P   L   A   V   I   L   V   M   Y   C   R   V   Y   I   V   A   K   R   T   T

AAG AAC CTA GAG GCA GGA GTC ATG AAG GAG ATG TCC AAC TCC AAG GAG CTG ACC CTG AGG ATC CAT TCC AAG AAC TTT
 K   N   L   E   A   G   V   M   K   E   M   S   N   S   K   E   L   T   L   R   I   H   S   K   N   F

CAC GAG GAC ACC CTT AGC AGT ACC AAG GCC AAG GGC CAC AAC CCC AGG AGT TCC ATA GCT GTC AAA CTT TTT AAG TTC
 H   E   D   T   L   S   S   T   K   A   K   G   H   N   P   R   S   S   I   A   V   K   L   F   K   F

TCC AGG GAA AAG AAA GCA GCT AAG ACG TTG GGC ATT GTG GTC GGT ATG TTC ATC TTG TGC TGG CTA CCC TTC TTC ATC
 S   R   E   K   K   A   A   K   T   L   G   I   V   V   G   M   F   I   L   C   W   L   P   F   F   I

GCT CTA CCG CTT GGC TCC TTG TTC TCC ACC CTG AAG CCC CCC GAC GCC GTG TTC AAG GTG GTG TTC TGG CTG GGC TAC
 A   L   P   L   G   S   L   F   S   T   L   K   P   P   D   A   V   F   K   V   V   F   W   L   G   Y

TTC AAC AGC TGC CTC AAC CCC ATC ATC TAC CCA TGC TCC AGC AAG GAG TTC AAG CGC GCT TTC GTG CGC ATC CTC GGG
 F   N   S   C   L   N   P   I   I   Y   P   C   S   S   K   E   F   K   R   A   F   V   R   I   L   G

TGC CAG TGC CGC GGC CGC GGC CGC CGC CGA CGC CGC CGC CGC CGT CGC CTG GGC GGC TGC GCC TAC ACC TAC CGG CCG
 C   Q   C   R   G   R   G   R   R   R   R   R   R   R   R   L   G   G   C   A   Y   T   Y   R   P

TGG ACG CGC GGC GGC TCG CTG GAG CGC TCG CAG TCG CGC AAG GAC TCG CTG GAC GAC AGC GGC AGC TGC CTG AGC GGC
 W   T   R   G   G   S   L   E   R   S   Q   S   R   K   D   S   L   D   D   S   G   S   C   L   S   G

AGC CAG CGG ACC CTG CCC TCG GCC TCG CCG AGC CCG GGC TAC CTG GGC CGC GGC GCG CCA CCG CCA GTC GAG CTG TGC
 S   Q   R   T   L   P   S   A   S   P   S   P   G   Y   L   G   R   G   A   P   P   P   V   E   L   C

GCC TTC CCC GAG TGG AAG GCG CCC GGC GCC CTC CTG AGC CTG CCC GCG CCT GAG CCC CCC GGC CGC CGC GGC CGC CAC
 A   F   P   E   W   K   A   P   G   A   L   L   S   L   P   A   P   E   P   P   G   R   R   G   R   H

GAC TCG GGC CCG CTC TTC ACC TTC AAG CTC CTG ACC GAG CCC GAG AGC CCC GGG ACC GAC GGC GGC GCC AGC AAC GGA
 D   S   G   P   L   F   T   F   K   L   L   T   E   P   E   S   P   G   T   D   G   G   A   S   N   G

GGC TGC GAG GCC GCG GCC GAC GTG GCC AAC GGG CAG CCG GGC TTC AAA AGC AAC ATG CCC CTG GCG CCC GGG CAG TTT
 G   C   E   A   A   A   D   V   A   N   G   Q   P   G   F   K   S   N   M   P   L   A   P   G   Q   F

TGA
Stp
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS158M	ChemiScreen™ Receptor Alpha1B Adrenergic Receptor Membrane Prep

REFERENCES

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2. Cavalli A *et al.* (1997) Decreased blood pressure response in mice deficient of the a_{1B}-AR. *Proc. Natl. Acad. Sci. USA* 94: 11589–11594

3. Milano CA *et al.* (1994) Myocardial expression of a constitutively active α_{1B}-adrenergic receptor in transgenic mice induces cardiac hypertrophy. *Proc. Natl. Acad. Sci. USA* 91: 10109-10113.

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