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PRODUCT DATASHEET

ChemiScreen™ α_{1A} Adrenergic Receptor Stable Cell Line

CATALOG NUMBER: HTS087C

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, α_{1A}, α_{1B} and $\alpha_{1D,}$ couple to G_q, 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; the α_{1A} subtype plays a prominent role in urogenital smooth muscle contraction and renal artery contraction (Hrometz *et al*., 1999; Ruffolo and Hieble, 1999). Activation of α1 adrenoceptors also influences cell proliferation; α_{1A} inhibits cell growth by arresting progression at the G_1/S transition (Shibata *et al.*, 2003). The α_{1A} subtype undergoes alternative splicing to generate four variants that differ at their C-termini, although these variants appear to be functionally identical (Chang *et al*., 1998). Cloned human α_{1A} receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-1 cells with α_{1A}. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the α_{1A} 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

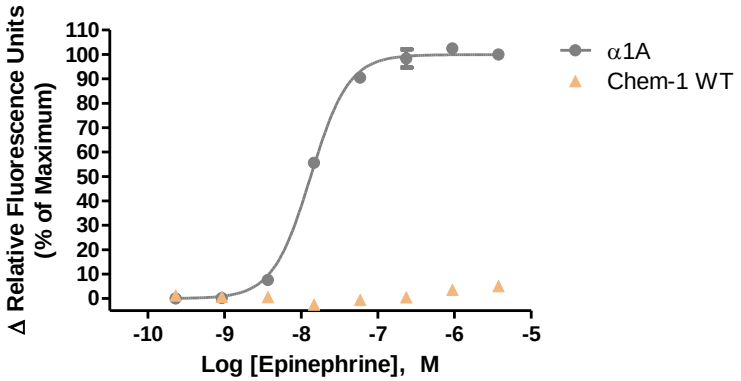

Figure 1. Representative data for activation of α_{1A} receptor stably expressed in Chem-1 cells induced by Epinephrine using a fluorescent calcium flux assay. α_{1A}–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a 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 13,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

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

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Epinephrine	Calcium Flux - Fluorescence	13	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 3µM 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™, 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

Human ADRA1A cDNA (Accession Number: NM_000680; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG GTG TTT CTC TCG GGA AAT GCT TCC GAC AGC TCC
                                                  M   V   F   L   S   G   N   A   S   D   S   S

AAC TGC ACC CAA CCG CCG GCA CCG GTG AAC ATT TCC AAG GCC ATT CTG CTC GGG GTG ATC TTG GGG GGC
 N   C   T   Q   P   P   A   P   V   N   I   S   K   A   I   L   L   G   V   I   L   G   G

CTC ATT CTT TTC GGG GTG CTG GGT AAC ATC CTA GTG ATC CTC TCC GTA GCC TGT CAC CGA CAC CTG CAC
 L   I   L   F   G   V   L   G   N   I   L   V   I   L   S   V   A   C   H   R   H   L   H

TCA GTC ACG CAC TAC TAC ATC GTC AAC CTG GCG GTG GCC GAC CTC CTG CTC ACC TCC ACG GTG CTG CCC
 S   V   T   H   Y   Y   I   V   N   L   A   V   A   D   L   L   L   T   S   T   V   L   P

TTC TCC GCC ATC TTC GAG GTC CTA GGC TAC TGG GCC TTC GGC AGG GTC TTC TGC AAC ATC TGG GCG GCA
 F   S   A   I   F   E   V   L   G   Y   W   A   F   G   R   V   F   C   N   I   W   A   A

GTG GAT GTG CTG TGC TGC ACC GCG TCC ATC ATG GGC CTC TGC ATC ATC TCC ATC GAC CGC TAC ATC GGC
 V   D   V   L   C   C   T   A   S   I   M   G   L   C   I   I   S   I   D   R   Y   I   G

GTG AGC TAC CCG CTG CGC TAC CCA ACC ATC GTC ACC CAG AGG AGG GGT CTC ATG GCT CTG CTC TGC GTC
 V   S   Y   P   L   R   Y   P   T   I   V   T   Q   R   R   G   L   M   A   L   L   C   V

TGG GCA CTC TCC CTG GTC ATA TCC ATT GGA CCC CTG TTC GGC TGG AGG CAG CCG GCC CCC GAG GAC GAG
 W   A   L   S   L   V   I   S   I   G   P   L   F   G   W   R   Q   P   A   P   E   D   E

ACC ATC TGC CAG ATC AAC GAG GAG CCG GGC TAC GTG CTC TTC TCA GCG CTG GGC TCC TTC TAC CTG CCT
 T   I   C   Q   I   N   E   E   P   G   Y   V   L   F   S   A   L   G   S   F   Y   L   P

CTG GCC ATC ATC CTG GTC ATG TAC TGC CGC GTC TAC GTG GTG GCC AAG AGG GAG AGC CGG GGC CTC AAG
 L   A   I   I   L   V   M   Y   C   R   V   Y   V   V   A   K   R   E   S   R   G   L   K

TCT GGC CTC AAG ACC GAC AAG TCG GAC TCG GAG CAA GTG ACG CTC CGC ATC CAT CGG AAA AAC GCC CCG
 S   G   L   K   T   D   K   S   D   S   E   Q   V   T   L   R   I   H   R   K   N   A   P

GCA GGA GGC AGC GGG ATG GCC AGC GCC AAG ACC AAG ACG CAC TTC TCA GTG AGG CTC CTC AAG TTC TCC
 A   G   G   S   G   M   A   S   A   K   T   K   T   H   F   S   V   R   L   L   K   F   S

CGG GAG AAG AAA GCG GCC AAA ACG CTG GGC ATC GTG GTC GGC TGC TTC GTC CTC TGC TGG CTG CCT TTT
 R   E   K   K   A   A   K   T   L   G   I   V   V   G   C   F   V   L   C   W   L   P   F

TTC TTA GTC ATG CCC ATT GGG TCT TTC TTC CCT GAT TTC AAG CCC TCT GAA ACA GTT TTT AAA ATA GTA
 F   L   V   M   P   I   G   S   F   F   P   D   F   K   P   S   E   T   V   F   K   I   V

TTT TGG CTC GGA TAT CTA AAC AGC TGC ATC AAC CCC ATC ATA TAC CCA TGC TCC AGC CAA GAG TTC AAA
 F   W   L   G   Y   L   N   S   C   I   N   P   I   I   Y   P   C   S   S   Q   E   F   K

AAG GCC TTT CAG AAT GTC TTG AGA ATC CAG TGT CTC CGC AGA AAG CAG TCT TCC AAA CAT GCC CTG GGC
 K   A   F   Q   N   V   L   R   I   Q   C   L   R   R   K   Q   S   S   K   H   A   L   G

TAC ACC CTG CAC CCG CCC AGC CAG GCC GTG GAA GGG CAA CAC AAG GAC ATG GTG CGC ATC CCC GTG GGA
 Y   T   L   H   P   P   S   Q   A   V   E   G   Q   H   K   D   M   V   R   I   P   V   G

TCA AGA GAG ACC TTC TAC AGG ATC TCC AAG ACG GAT GGC GTT TGT GAA TGG AAA TTT TTC TCT TCC ATG
 S   R   E   T   F   Y   R   I   S   K   T   D   G   V   C   E   W   K   F   F   S   S   M

CCC CGT GGA TCT GCC AGG ATT ACA GTG TCC AAA GAC CAA TCC TCC TGT ACC ACA GCC CGG GTG AGA AGT
 P   R   G   S   A   R   I   T   V   S   K   D   Q   S   S   C   T   T   A   R   V   R   S

AAA AGC TTT TTG CAG GTC TGC TGC TGT GTA GGG CCC TCA ACC CCC AGC CTT GAC AAG AAC CAT CAA GTT
 K   S   F   L   Q   V   C   C   C   V   G   P   S   T   P   S   L   D   K   N   H   Q   V

CCA ACC ATT AAG GTC CAC ACC ATC TCC CTC AGT GAG AAC GGG GAG GAA GTC TAG TGA
 P   T   I   K   V   H   T   I   S   L   S   E   N   G   E   E   V     STP
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 host parental cell line (control cells)
HTS087M	ChemiScreen™ α_{1A} Adrenergic receptor membrane prep

REFERENCES

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3. Hrometz SL *et al.* (1999) Expression of multiple alpha1-adrenoceptors on vascular smooth muscle: correlation with the regulation of contraction. *J. Pharmacol. Exp. Ther.* 290(1):452-63.
4. Ruffolo JR RR and Hieble JP (1999) Adrenoceptor pharmacology: urogenital applications. *Eur. Urol.* 36 (suppl. 1): 17-22.
5. Shibata K *et al.* (2003) α_1-Adrenergic receptor subtypes differentially control the cell cycle of transfected CHO cells through a cAMP-dependent mechanism involving $p27^{Kip1}$. *J. Biol. Chem.* 278: 672-678.

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