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PRODUCT DATASHEET

ChemiScreen™ α1D Adrenergic Receptor Stable Cell Line

CATALOG NUMBER: HTS216C

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 α_1 subclass of adrenoceptors, α_{1A}, α_{1B} and α_{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_{1D} adrenergic receptor mediates smooth muscle contraction in several tissues. In the vasculature, activation of α_{1D} increases blood pressure (Tanoue et al., 2002; Hosoda et al., 2005). In the urinary tract, α_{1D} promotes bladder contraction. Antagonists of α_1 receptors are used to treat bladder outlet obstruction, and this effect is thought to be mediated by α_{1D} (Chen et al., 2005). The α_{1D} adrenergic receptors have a relatively long N-terminal extracellular domain, and truncation of this domain has been shown to increase expression of the receptor at the cell surface (Pupo et al., 2003). The cloned human α_{1D} -expressing cell line contains a version of α_{1D} lacking residues 2-79. The cell line is made in the Chem-1 host, which supports high levels of recombinant α_{1D} 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 α_{1D} and its ligands.

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

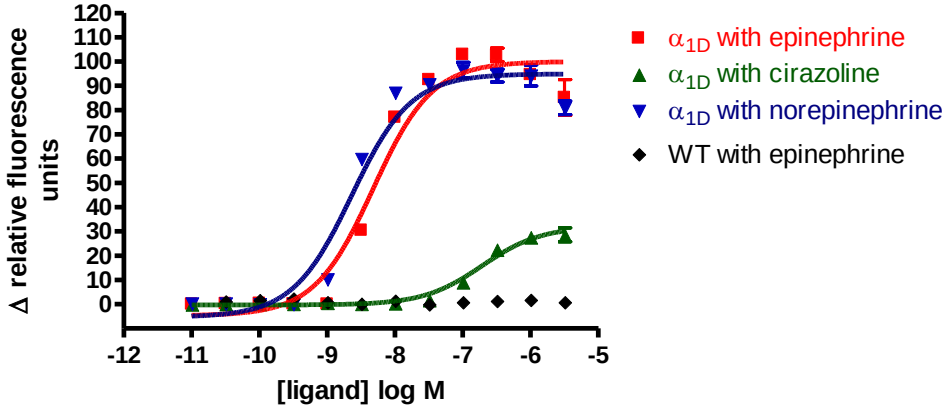

Figure 1. Representative data for activation of the α_{1D} receptor stably expressed in Chem-1 cells induced by epinephrine using a fluorescent calcium flux assay. α_{1D} –expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with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

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

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Epinephrine	Calcium Flux - Fluorescence	4.8	Eurofins Internal Data
Cirazoline	Calcium Flux - Fluorescence	190	Eurofins Internal Data
Norepinephrine	Calcium Flux - Fluorescence	2.3	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 Epinephrine, was 0.84.

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	10mM	Millipore Sigma: H3537
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Gibco:10131-027
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	Sigma: E1635
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

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 by quickly inverting plate on an absorbent pad and blotting to remove all Media Component.
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 ADRA1D cDNA encoding α_{1D} lacking residues 2-79 (Accession Number: NM_000678) and promiscuous G protein are expressed in a bicistronic vector.

RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS216M	ChemiScreen™ Receptor ADRA1D 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. Chen Q *et al*. (2005) Function of the lower urinary tract in mice lacking α_{1d}-adrenoceptor. *J. Urol.* 174: 370-374.

3. Horie K *et al.* (1995) Selectivity of the imidazoline α-adrenoceptor agonists (oxymetazoline and cirazoline) for human cloned α_1-adrenoceptor subtypes. *Br. J. Pharmacol.* 116: 1611-1618.

4. Hosoda C *et al*. (2005) Two α_1-adrenergic receptor subtypes regulating the vasopressor response have differential roles in blood pressure regulation. *Mol. Pharmacol.* 67: 912-922.

5. Pupo AS *et al.* (2003) N-terminal truncation of human α_{1D}-adrenoceptors increases expression of binding sites but not protein. *Eur. J. Pharmacol.* 462: 1-8.

6. Tanoue A *et al.* (2002) The α_{1D}-adrenergic receptor directly regulates arterial blood pressure via vasoconstriction. *J. Clin. Invest.* 109: 765-775.

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