eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ α_{1A} Adrenergic Receptor Frozen Cells

CATALOG NUMBER: HTS087RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

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 ▪- and ▪-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 α_1 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} -expressing cell line is made in the Chem-1 host, which supports high levels of recombinant α_{1A} expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening agonists, antagonists and modulators at α_{1A} adrenergic receptors.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

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 Assays

APPLICATION DATA

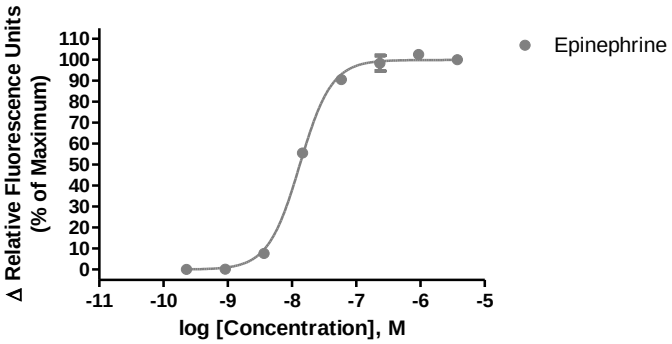

Figure 1. Representative data for activation of α_{1A} receptor. Calcium flux in α_{1A} -expressing Chem-1 cell line induced by Epinephrine. α_{1A} –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand, 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 20,000 RLU (Relative Light Units).

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

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Epinephrine	Calcium Flux	13	Eurofins Internal Data

ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.

10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm ($FLIPR^{TETRA}$) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Epinephrine ligand	Sigma: E1635
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

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	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS GENE EXPRESSION

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-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS087M	ChemiScreen™ α1A Adrenergic receptor membrane prep

REFERENCES

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2. Chang DJ *et al.* (1998) Molecular cloning, genomic characterization and expression of novel human α1A-adrenoceptor isoforms. *FEBS Lett.* 422: 279-83.
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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