eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ α_{2A} Adrenergic Family Receptor Frozen Cells

CATALOG NUMBER: HTS096RTA

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 α- and β-adrenoceptors (Bylund et al., 1994). The α_2 adrenergic receptor subfamily members, consisting of α_{2A}, α_{2B}, and α_{2C}, couple primarily to Gi to inhibit cAMP production, and play an important role in regulation of cardiovascular and CNS function. The α_{2A} receptor at presynaptic sites has an inhibitory effect on catecholamine release from sympathetic nerve endings. Experiments with α_{2A} -selective agonists and mice lacking α_{2A} demonstrate that activation of α_{2A} results in hypotension, sedation, analgesia, and hypothermia (Kable et al., 2000). Cloned human α_{2A} -expressing cell line is made in the Chem-1 host, which supports high levels of recombinant α_{2A} expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at α_{2A}.

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

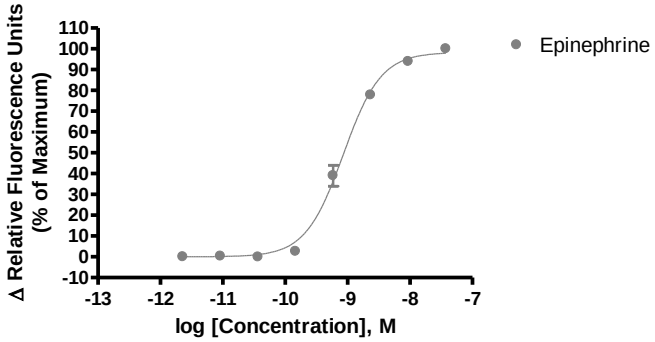

Figure 1. Representative data for activation of α_{2A} receptor. Calcium flux in α_{2A} –expressing Chem-1 cell line induced by epinephrine. α_{2A} –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 4,000 RLU (Relative Light Units).

Table 1. EC_{50} values of α_{2A}-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Epinephrine	Calcium Flux	0.9	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

ADRA2A cDNA (Accession Number: NM_000681; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG GGC TCC CTG CAG CCG GAC GCG GGC AAC GCG AGC
                                                 M   G   S   L   Q   P   D   A   G   N   A   S

TGG AAC GGG ACC GAG GCG CCG GGG GGC GGC GCC CGG GCC ACC CCT TAC TCC CTG CAG GTG ACG CTG ACG
 W   N   G   T   E   A   P   G   G   G   A   R   A   T   P   Y   S   L   Q   V   T   L   T

CTG GTG TGC CTG GCC GGC CTG CTC ATG CTG CTC ACC GTG TTC GGC AAC GTG CTC GTC ATC ATC GCC GTG
 L   V   C   L   A   G   L   L   M   L   L   T   V   F   G   N   V   L   V   I   I   A   V

TTC ACG AGC CGC GCG CTC AAG GCG CCC CAA AAC CTC TTC CTG GTG TCT CTG GCC TCG GCC GAC ATC CTG
 F   T   S   R   A   L   K   A   P   Q   N   L   F   L   V   S   L   A   S   A   D   I   L

GTG GCC ACG CTC GTC ATC CCT TTC TCG CTG GCC AAC GAG GTC ATG GGC TAC TGG TAC TTC GGC AAG GCT
 V   A   T   L   V   I   P   F   S   L   A   N   E   V   M   G   Y   W   Y   F   G   K   A

TGG TGC GAG ATC TAC CTG GCG CTC GAC GTG CTC TTC TGC ACG TCG TCC ATC GTG CAC CTG TGC GCC ATC
 W   C   E   I   Y   L   A   L   D   V   L   F   C   T   S   S   I   V   H   L   C   A   I

AGC CTG GAC CGC TAC TGG TCC ATC ACA CAG GCC ATC GAG TAC AAC CTG AAG CGC ACG CCG CGC CGC ATC
 S   L   D   R   Y   W   S   I   T   Q   A   I   E   Y   N   L   K   R   T   P   R   R   I

AAG GCC ATC ATC ATC ACC GTG TGG GTC ATC TCG GCC GTC ATC TCC TTC CCG CCG CTC ATC TCC ATC GAG
 K   A   I   I   I   T   V   W   V   I   S   A   V   I   S   F   P   P   L   I   S   I   E

AAG AAG GGC GGC GGC GGC GGC CCG CAG CCG GCC GAG CCG CGC TGC GAG ATC AAC GAC CAG AAG TGG TAC
 K   K   G   G   G   G   G   P   Q   P   A   E   P   R   C   E   I   N   D   Q   K   W   Y

GTC ATC TCG TCG TGC ATC GGC TCC TTC TTC GCT CCC TGC CTC ATC ATG ATC CTG GTC TAC GTG CGC ATC
 V   I   S   S   C   I   G   S   F   F   A   P   C   L   I   M   I   L   V   Y   V   R   I

TAC CAG ATC GCC AAG CGT CGC ACC CGC GTG CCA CCC AGC CGC CGG GGT CCG GAC GCC GTC GCC GCG CCG
 Y   Q   I   A   K   R   R   T   R   V   P   P   S   R   R   G   P   D   A   V   A   A   P

CCG GGG GGC ACC GAG CGC AGG CCC AAC GGT CTG GGC CCC GAG CGC AGC GCG GGC CCG GGG GGC GCA GAG
 P   G   G   T   E   R   R   P   N   G   L   G   P   E   R   S   A   G   P   G   G   A   E

GCC GAA CCG CTG CCC ACC CAG CTC AAC GGC GCC CCT GGC GAG CCC GCG CCG GCC GGG CCG CGC GAC ACC
 A   E   P   L   P   T   Q   L   N   G   A   P   G   E   P   A   P   A   G   P   R   D   T

GAC GCG CTG GAC CTG GAG GAG AGC TCG TCT TCC GAC CAC GCC GAG CGG CCT CCA GGG CCC CGC AGA CCC
 D   A   L   D   L   E   E   S   S   S   S   D   H   A   E   R   P   P   G   P   R   R   P

GAG CGC GGT CCC CGG GGC AAA GGC AAG GCC CGA GCG AGC CAG GTG AAG CCG GGC GAC AGC CTG CCG CGG
 E   R   G   P   R   G   K   G   K   A   R   A   S   Q   V   K   P   G   D   S   L   P   R

CGC GGG CCG GGG GCG ACG GGG ATC GGG ACG CCG GCT GCA GGG CCG GGG GAG GAG CGC GTC GGG GCT GCC
 R   G   P   G   A   T   G   I   G   T   P   A   A   G   P   G   E   E   R   V   G   A   A

AAG GCG TCG CGC TGG CGC GGG CGG CAG AAC CGC GAG AAG CGC TTC ACG TTC GTG CTG GCC GTG GTC ATC
 K   A   S   R   W   R   G   R   Q   N   R   E   K   R   F   T   F   V   L   A   V   V   I

GGA GTG TTC GTG GTG TGC TGG TTC CCC TTC TTC TTC ACC TAC ACG CTC ACG GCC GTC GGG TGC TCC GTG
 G   V   F   V   V   C   W   F   P   F   F   F   T   Y   T   L   T   A   V   G   C   S   V

CCA CGC ACG CTC TTC AAA TTC TTC TTC TGG TTC GGC TAC TGC AAC AGC TCG TTG AAC CCG GTC ATC TAC
 P   R   T   L   F   K   F   F   F   W   F   G   Y   C   N   S   S   L   N   P   V   I   Y

ACC ATC TTC AAC CAC GAT TTC CGC CGC GCC TTC AAG AAG ATC CTC TGT CGG GGG GAC AGG AAG CGG ATC
 T   I   F   N   H   D   F   R   R   A   F   K   K   I   L   C   R   G   D   R   K   R   I

GTG TGA
 V  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS096M	ChemiScreen™ α_{2A} adrenergic family receptor membrane prep

REFERENCES

1. Bylund DB *et al.* (1994). IV. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.* 46: 121-136.
2. Kable JW *et al.* (2000) In vivo gene modification elucidates subtype-specific functions of ·$_2$-adrenergic receptors. *J. Pharmacol. Exp. Ther.* 293: 1-7.

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