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PRODUCT DATASHEET

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

CATALOG NUMBER: HTS216RTA

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 three members of the α_1 subclass of adrenoceptors, α_{1A}, α_{1B} 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. 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 receptor has 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). 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 agonists and antagonists at α_{1D}.

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

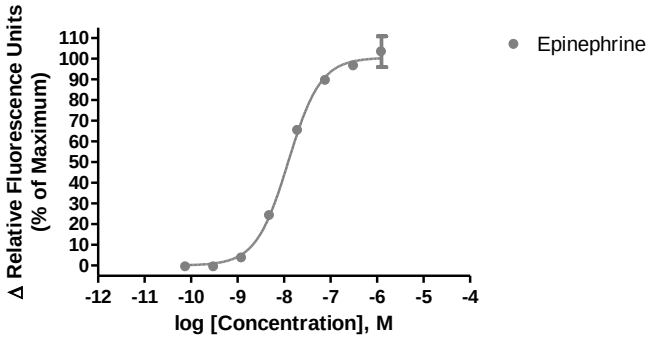

Figure 1. Representative data for activation of α_{1D} receptor. Calcium flux in α_{1D}–expressing Chem-1 cell line induced by Epinephrine. α_{1D}–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,300 RLU (Relative Light Units).

Table 1. Comparison of EC_{50} values of α_{1D}-expressing Chem-1 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Epinephrine	Calcium Flux	12	Eurofins Internal Data
Norepinephrine	Calcium Flux	3	Horie et al., 1995

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.

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EXONGENOUS GENE EXPRESSION

ADRA1D cDNA (Accession Number: NM_000678 with N-term truncation; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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  1 - ATG ACT TTC CGC GAT CTC CTG AGC GTC AGT TTC GAG GGA CCC CGC CCG GAC AGC AGC GCA GGG GGC TCC AGC  - 72
  1 -  M   T   F   R   D   L   L   S   V   S   F   E   G   P   R   P   D   S   S   A   G   G   S   S  - 24

 73 - CGC GGC CGC GCC CGG CGC ACG CCG CGC CGC CCG CCG CCC TCG CAG CGC CCG CCG GTG CGC CGC GTG CCG CGC  - 144
 25 -  A   G   G   G   G   S   A   G   G   A   A   P   S   E   G   P   A   V   G   G   V   P   G   - 48

145 - CGC GCC CGC CGC CGC GCC CGC CTG CTG CGC CCA CGC AGC CGC CAG CAC AAC CGG ACG TCC CCG CGG GAG CCG  - 216
 49 -  G   A   G   G   G   G   G   V   V   G   A   G   S   G   E   D   N   R   S   S   A   G   E   P  - 72

217 - GGG AGC GCG GGC GCG GGC GGC GAC GTG AAT GGC ACG GCG GCC GTC GGG GGA CTG GTG GTG AGC GCG CAG GGC  - 288
 73 -  G   S   A   G   A   G   G   D   V   N   G   T   A   A   V   G   G   L   V   V   S   A   Q   G  - 96

289 - GTG GGC GTG GGC GTC TTC CTG GCA GCC TTC ATC CTT ATG GCC GTG GCA GGT AAC CTG CTT GTC ATC CTC TCA  - 360
 97 -  V   G   V   G   V   F   L   A   A   F   I   L   M   A   V   A   G   N   L   L   V   I   L   S  - 120

361 - GTG GCC TGC AAC CGC CAC CTG CAG ACC GTC ACC AAC TAT TTC ATC GTG AAC CTG GCC GTG GCC GAC CTG CTG  - 432
121 -  V   A   C   N   R   H   L   Q   T   V   T   N   Y   F   I   V   N   L   A   V   A   D   L   L  - 144

433 - CTG AGC GCC ACC GTA CTG CCC TTC TCG GCC ACC ATG GAG GTT CTG GGC TTC TGG GCC TTT GGC CGC GCC TTC  - 504
145 -  L   S   A   T   V   L   P   F   S   A   T   M   E   V   L   G   F   W   A   F   G   R   A   F  - 168

505 - TGC GAC GTA TGG GCC GCC GTG GAC GTG CTG TGC TGC ACG GCC TCC ATC CTC AGC CTC TGC ACC ATC TCC GTG  - 576
169 -  C   D   V   W   A   A   V   D   V   L   C   C   T   A   S   I   L   S   L   C   T   I   S   V  - 192

577 - GAC CGG TAC GTG GGC GTG CGC CAC TCA CTC AAG TAC CCA GCC ATC ATG ACC GAG CGC AAG GCG GCC GCC ATC  - 648
193 -  D   R   Y   V   G   V   R   H   S   L   K   Y   P   A   I   M   T   E   R   K   A   A   A   I  - 216

649 - CTG GCC CTG CTC TGG GTC GTA GCC CTG GTG GTG TCC GTA GGG CCC CTG CTG GGC TGG AAG GAG CCC GTG CCC  - 720
217 -  L   A   L   L   W   V   V   A   L   V   V   S   V   G   P   L   L   G   W   K   E   P   V   P  - 240

721 - CCT GAC GAG CGC TTC TGC GGT ATC ACC GAG GAG GCG GGC TAC GCT GTC TTC TCC TCC GTG TGC TCC TTC TAC  - 792
241 -  P   D   E   R   F   C   G   I   T   E   E   A   G   Y   A   V   F   S   S   V   C   S   F   Y  - 264

793 - CTG CCC ATG GCG GTC ATC GTG GTC ATG TAC TGC CGC GTG TAC GTG GTC GCG CGC AGC ACC ACG CGC AGC CTC  - 864
265 -  L   P   M   A   V   I   V   V   M   Y   C   R   V   Y   V   V   A   R   S   T   T   R   S   L  - 288

865 - GAG GCG GGC GTC AAG CGC GAG CGA GGC AAG GCC TCC GAG GTG GTG CTG CGC ATC CAC TGT CGC GGC GCG GCC  - 936
289 -  E   A   G   V   K   R   E   R   G   K   A   S   E   V   V   L   R   I   H   C   R   G   A   A  - 312

937 - ACG GGC GCC GAC GGG GCA CAC GGC ATG CGC AGC GCC AAG GGC CAC ACC TTC CGC AGC TCG CTC TCC GTG CGC  - 1008
313 -  T   G   A   D   G   A   H   G   M   R   S   A   K   G   H   T   F   R   S   S   L   S   V   R  - 336

1009 - CTG CTC AAG TTC TCC CGT GAG AAG AAA GCG GCC AAG ACT CTG GCC ATC GTC GTG GGT GTC TTT GTG CTC TGC  - 1080
337 -  L   L   K   F   S   R   E   K   K   A   A   K   T   L   A   I   V   V   G   V   F   V   L   C  - 360

1081 - TGG TTC CCT TTC TTC TTT GTC CTG CCG CTC GGC TCC TTG TTC CCG CAG CTG AAG CCA TCG GAG GGC GTC TTC  - 1152
361 -  W   F   P   F   F   F   V   L   P   L   G   S   L   F   P   Q   L   K   P   S   E   G   V   F  - 384

1153 - AAG GTC ATC TTC TGG CTC GGC TAC TTC AAC AGC TGC GTG AAC CCG CTC ATC TAC CCC TGT TCC AGC CGC GAG  - 1224
385 -  K   V   I   F   W   L   G   Y   F   N   S   C   V   N   P   L   I   Y   P   C   S   S   R   E  - 408

1225 - TTC AAG CGC GCC TTC CTC CGT CTC CTG CGC TGC CAG TGC CGT CGT CGC CGG CGC CGC CGC CCT CTC TGG CGT  - 1296
409 -  F   K   R   A   F   L   R   L   L   R   C   Q   C   R   R   R   R   R   R   R   P   L   W   R  - 432

1297 - GTC TAC GGC CAC CAC TGG CGG GCC TCC ACC AGC GGC CTG CGC CAG GAC TGC GCC CCG AGT TCG GGC GAC GCG  - 1368
433 -  V   Y   G   H   H   W   R   A   S   T   S   G   L   R   Q   D   C   A   P   S   S   G   D   A  - 456

1369 - CCC CCC GGA GCG CCG CTG GCC CTC ACC GCG CTC CCC GAC CCC GAC CCC GAA CCC CCA GGC ACG CCC GAG ATG  - 1440
457 -  P   P   G   A   P   L   A   L   T   A   L   P   D   P   D   P   E   P   P   G   T   P   E   M  - 480

1441 - CAG GCT CCG GTC GCC AGC CGT CGA AAG CCA CCC AGC GCC TTC CGC GAG TGG AGG CTG CTG GGG CCA TTC CGG  - 1512
481 -  Q   A   P   V   A   S   R   R   K   P   P   S   A   F   R   E   W   R   L   L   G   P   F   R  - 504

1513 - AGA CCC ACG ACC CAG CTG CGC GCC AAA GTC TCC AGC CTG TCG CAC AAG ATC CGC GCC GGG GGC GCG CAG CGC  - 1584
505 -  R   P   T   T   Q   L   R   A   K   V   S   S   L   S   H   K   I   R   A   G   G   A   Q   R  - 528

1585 - GCA GAG GCA GCG TGC GCC CAG CGC TCA GAG GTG GAG GCT GTG TCC CTA GGC GTC CCA CAC GAG GTG GCC GAG  - 1656
529 -  A   E   A   A   C   A   Q   R   S   E   V   E   A   V   S   L   G   V   P   H   E   V   A   E  - 552

1657 - GGC GCC ACC TGC CAG GCC TAC GAA TTG GCC GAC TAC AGC AAC CTA CGG GAG ACC GAT ATT TAA
          553 -  G   A   T   C   Q   A   Y   E   L   A   D   Y   S   N   L   R   E   T   D   I  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS216M	ChemiScreen™ α1D 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. 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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