eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ ET_B Endothelin Receptor Frozen Cells

CATALOG NUMBER: HTS046RTA

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 endothelin family of peptides are potent vasoconstrictors synthesized by endothelial cells in both constitutive and inducible pathways. The biological actions of endothelins are mediated by two GPCRs, ET_A and ET_B (Davenport, 2002). Whereas ET_A is the predominant receptor on smooth muscle cells and thus is the primary mediator of the vasoconstrictor activity, ET_B is found on endothelial cells and mediates vasodilation (Nilsson *et al.*, 1997). In addition, ET_B has also been linked to renal failure, congestive heart failure, atherosclerosis, and pulmonary hypertension (D'Orléans-Juste, *et al.,* 2002). A mutation in ET_B has been found to cause Hirschsprung disease-2, a degenerative disease of the digestive tract (Puffenberger *et al*, 1994). Cloned human ET_B-expressing cell line is made in the CHO host, which supports high levels of recombinant ET_B expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists, and modulators at ET_B.

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

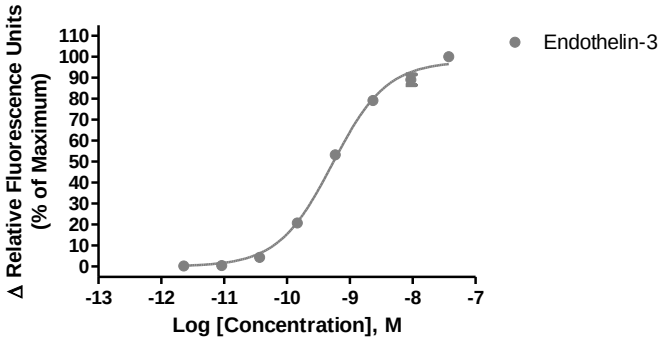

Figure 1. Representative data for activation of ET_B receptor. Calcium flux in ET_B–expressing CHO cell line induced by Endothelin-3. ET_B–expressing CHO cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 1,600 RLU (Relative Light Units).

Table 1. EC_{50} values of ET_B-expressing CHO cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Endothelin-3	Calcium Flux	0.5	Eurofins Internal Data
Endothelin-1	Calcium Flux	0.2	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
Endothelin-3 ligand	Tocris: 1162
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

Chinese Hamster Ovarian K-1 cells (CHO K-1).

EXONGENOUS GENE EXPRESSION

EDNRB cDNA (Accession Number: NM_000115; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                        ATG CAG CCG CCT CCA AGT CTG
                                                         M   Q   P   R   P   S   L

TGC GGA CGC GCC CTG GTT GCG CTG GTT CTT GCC TGC GGC CTG TCG CGG ATC TGG GGA GAG
 C   G   R   A   L   V   A   L   V   L   A   C   G   L   S   R   I   W   G   E

GAG AGA GGC TTC CCG CCT GAC AGG GCC ACT CCG CTT TTG CAA ACC GCA GAG ATA ATG ACG
 E   R   G   F   P   P   D   R   A   T   P   L   L   Q   T   A   E   I   M   T

CCA CCC ACT AAG ACC TTA TGG CCC AAG GGT TCC AAC GCC AGT CTG GCG CGG TCG TTG GCA
 P   P   T   K   T   L   W   P   K   G   S   N   A   S   L   A   R   S   L   A

CCT GCG GAG GTG CCT AAA GGA GAC AGG ACG GCA GGA TCT CCG CCA CGC ACC ATC TCC CCT
 P   A   E   V   P   K   G   D   R   T   A   G   S   P   P   R   T   I   S   P

CCC CCG TGC CAA GGA CCC ATC GAG ATC AAG GAG ACT TTC AAA TAC ATC AAC ACG GTT GTG
 P   P   C   Q   G   P   I   E   I   K   E   T   F   K   Y   I   N   T   V   V

TCC TGC CTT GTG TTC GTG CTG GGG ATC ATC GGG AAC TCC ACA CTT CTG AGA ATT ATC TAC
 S   C   L   V   F   V   L   G   I   I   G   N   S   T   L   L   R   I   I   Y

AAG AAC AAG TGC ATG CGA AAC GGT CCC AAT ATC TTG ATC GCC AGC TTG GCT CTG GGA GAC
 K   N   K   C   M   R   N   G   P   N   I   L   I   A   S   L   A   L   G   D

CTG CTG CAC ATC GTC ATT GAC ATC CCT ATC AAT GTC TAC AAG CTG CTG GCA GAG GAC TGG
 L   L   H   I   V   I   D   I   P   I   N   V   Y   K   L   L   A   E   D   W

CCA TTT GGA GCT GAG ATG TGT AAG CTG GTG CCT TTC ATA CAG AAA GCC TCC GTG GGA ATC
 P   F   G   A   E   M   C   K   L   V   P   F   I   Q   K   A   S   V   G   I

ACT GTG CTG AGT CTA TGT GCT CTG AGT ATT GAC AGA TAT CGA GCT GTT GCT TCT TGG AGT
 T   V   L   S   L   C   A   L   S   I   D   R   Y   R   A   V   A   S   W   S

AGA ATT AAA GGA ATT GGG GTT CCA AAA TGG ACA GCA GTA GAA ATT GTT TTG ATT TGG GTG
 R   I   K   G   I   G   V   P   K   W   T   A   V   E   I   V   L   I   W   V

GTC TCT GTG GTT CTG GCT GTC CCT GAA GCC ATA GGT TTT GAT ATA ATT ACG ATG GAC TAC
 V   S   V   V   L   A   V   P   E   A   I   G   F   D   I   I   T   M   D   Y

AAA GGA AGT TAT CTG CGA ATC TGC TTG CTT CAT CCC GTT CAG AAG ACA GCT TTC ATG CAG
 K   G   S   Y   L   R   I   C   L   L   H   P   V   Q   K   T   A   F   M   Q

TTT TAC AAG ACA GCA AAA GAT TGG TGG CTG TTC AGT TTC TAT TTC TGC TTG CCA TTG GCC
 F   Y   K   T   A   K   D   W   W   L   F   S   F   Y   F   C   L   P   L   A

ATC ACT GCA TTT TTT TAT ACA CTA ATG ACC TGT GAA ATG TTG AGA AAG AAA AGT GGC ATG
 I   T   A   F   F   Y   T   L   M   T   C   E   M   L   R   K   K   S   G   M

CAG ATT GCT TTA AAT GAT CAC CTA AAG CAG AGA CGG GAA GTG GCC AAA ACC GTC TTT TGC
 Q   I   A   L   N   D   H   L   K   Q   R   R   E   V   A   K   T   V   F   C

CTG GTC CTT GTC TTT GCC CTC TGC TGG CTT CCC CTT CAC CTC AGC AGG ATT CTG AAG CTC
 L   V   L   V   F   A   L   C   W   L   P   L   H   L   S   R   I   L   K   L

ACT CTT TAT AAT CAG AAT GAT CCC AAT AGA TGT GAA CTT TTG AGC TTT CTG TTG GTA TTG
 T   L   Y   N   Q   N   D   P   N   R   C   E   L   L   S   F   L   L   V   L

GAC TAT ATT GGT ATC AAC ATG GCT TCA CTG AAT TCC TGC ATT AAC CCA ATT GCT CTG TAT
 D   Y   I   G   I   N   M   A   S   L   N   S   C   I   N   P   I   A   L   Y

TTG GTG AGC AAA AGA TTC AAA AAC TGC TTT AAG TCA TGC TTA TGC TGC TGG TGC CAG TCA
 L   V   S   K   R   F   K   N   C   F   K   S   C   L   C   C   W   C   Q   S

TTT GAA GAA AAA CAG TCC TTG GAG GAA AAG CAG TCG TGC TTA AAG TTC AAA GCT AAT GAT
 F   E   E   K   Q   S   L   E   E   K   Q   S   C   L   K   F   K   A   N   D

CAC GGA TAT GAC AAC TTC CGT TCC AGT AAT AAA TAC AGC TCA TCT TGA
 H   G   Y   D   N   F   R   S   S   N   K   Y   S   S   S   St
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTS046M	ChemiScreen™ ET_B Endothelin receptor membrane prep

REFERENCES

1. Davenport AP (2002) International Union of Pharmacology. XXIX. Update on Endothelin Receptor Nomenclature. *Pharmacol. Rev.* 54: 219-226.
2. Nilsson T, *et al.* (1997) Presence of contractile endothelin-A and dilatory endothelin-B receptors in human cerebral arteries. *Neurosurgery.* 40: 346–351;comment 351–353.
3. D'Orléans-Juste P, *et al* (2002) Function of the $endothelin_B$ receptor in cardiovascular physiology and pathophysiology. *Pharmacol Ther.* 95: 221-238.
4. Puffenberger EG, *et al* (1994) A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. *Cell* 79: 1257-1266.

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