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PRODUCT DATASHEET

Ready-to-Assay™ $VPAC_1$ VIP and PACAP Receptor Frozen Cells

CATALOG NUMBER: HTS043RTA

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.

Vasoactive intestinal peptide (VIP), a 28 amino acid peptide originally isolated by its vasodilation activity, binds to two class B GPCRs, VPAC1 and VPAC2, to exert its functions in the CNS, vasculature, immune system and adrenal medulla (Harmar *et al.*, 1998). In the immune system, VIP is synthesized by mast cells and lymphocytes, and appears to inhibit inflammation and to shift the immune response toward a Th2 pathway (Delgado *et al.*, 2004). In the heart, VIP is expressed by nerve fibers, where it modulates heart rate, and coronary blood flow (Henning and Sawmiller, 2001). Cloned human $VPAC_1$-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant $VPAC_1$ 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 $VPAC_1$.

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

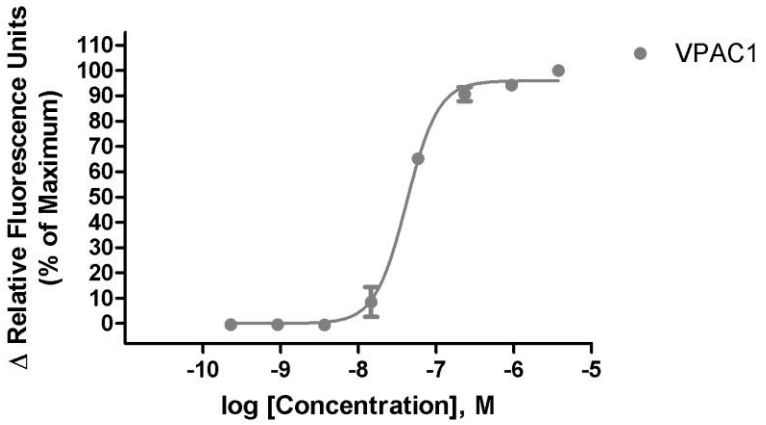

Figure 1. Representative data for activation of $VPAC_1$ receptor. Calcium flux in $VPAC_1$-expressing Chem-1 cell line induced by VIP. $VPAC_1$–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,400 RLU (Relative Light Units).

Table 1. Comparison of EC50 values of VPAC¬1-expressing Chem-1 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
VIP	Calcium Flux	40	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
VIP ligand	Sigma: V6130
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

VIPR cDNA (Accession Number: NM_004624; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary pHS plasmid.

CODING SEQUENCE

ATG CGC CCG CCA AGT CCG CTG CCC GCC CGC TGG CTA TGC GTG CTG GCA GGC GCC CTC GCC TGG

GCC CTT GGG CCG GCG GGC GGC CAG GCG GCC AGG CTG CAG GAG GAG TGT GAC TAT GTG CAG ATG

ATC GAG GTG CAG CAC AAG CAG TGC CTG GAG GAG GCC CAG CTG GAG AA**C** GAG ACA ATA GGC TGC

AGC AAG ATG TGG GAC AAC CTC ACC TGC TGG CCA GCC ACC CCT CGG GGC CAG GTA GTT GTC TTG

GCC TGT CCC CTC ATC TTC AAG CTC TTC TCC TCC ATT CAA GGC CGC AAT GTA AGC CGC AGC TGC

ACC GAC GAA GGC TGG ACG CAC CTG GAG CCT GGC CCG TAC CCC ATT GCC TGT GGT TTG GAT GAC

AAG GCA GCG AGT TTG GAT GAG CAG CAG ACC ATG TTC TAC GGT TCT GTG AAG ACC GGC TAC ACC

ATC GGC TAC GGC CTG TCC CTC GCC ACC CTT CTG GTC GCC ACA GCT ATC CTG AGC CTG TTC AGG

AAG CTC CAC TGC ACG CGG AAC TAC ATC CAC ATG CAC CTC TTC ATA TCC TTC ATC CTG AGG GCT

GCC GCT GTC TTC ATC AAA GAC TTG GCC CTC TTC GAC AGC GGG GAG TCG GAC CAG TGC TCC GAG

GGC TCG GTG GGC TGT AAG GCA GCC ATG GTC TTT TTC CAA TAT TGT GTC ATG GCT AAC TTC TTC

TGG CTG CTG GTG GAG GGC CTC TAC CTG TAC ACC CTG CTT GCC GTC TCC TTC TTC TCT GAG CGG

AAG TAC TTC TGG GGG TAC ATA CTC ATC GGC TGG GGG GTA CCC AGC ACA TTC ACC ATG GTG TGG

ACC ATC GCC AGG ATC CAT TTT GAG GAT TAT GGG TGC TGG GAC ACC ATC AAC TCC TCA CTG TGG

TGG ATC ATA AAG GGC CCC ATC CTC ACC TCC ATC TTG GTA AAC TTC ATC CTG TTT ATT TGC ATC

ATC CGA ATC CTG CTT CAG AAA CTG CGG CCC CCA GAT ATC AGG AAG AGT GAC AGC AGT CCA TAC

TCA AGG CTA GCC A**T**G TCC ACA CTC CTG CTG ATC CCC CTG TTT GGA GTA CAC TAC ATC ATG TTC

GCC TTC TTT CCG GAC AAT TTT AAG CCT GAA GTG AAG ATG GTC TTT GAG CTC GTC GTG GGG TCT

TTC CAG GGT TTT GTG GTG GCT ATC CTC TAC TGC TTC CTC AAT GGT GAG GTG CAG GCG GAG CTG

AGG CGG AAG TGG CGG CGC TGG CAC CTG CAG GGC GTC CTG GGC TGG AAC CCC AAA TAC CGG CAC

CCG TCG GGA GGC AGC AAC GGC GCC ACG TGC AGC ACG CAG GTT TCC ATG CTG ACC CGC GTC AGC

CCA GGT GCC CGC CGC TCC TCC AGC TTC CAA GCC GAA GTC TCC CTG GTC TAA

RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS043M	ChemiScreen™ VPAC1 VIP and PACAP receptor membrane prep

REFERENCES

1. Delgado M *et al.* (2004) The significance of vasoactive intestinal peptide in immunomodulation. *Pharmacol. Rev.* 56: 249-290.
2. Harmar AJ *et al.* (1998) International Union of Pharmacology. XVIII. Nomenclature of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. *Pharmacol. Rev.* 50: 265-270.
3. Henning RJ and Sawmiller DR (2001) Vasoactive intestinal peptide: cardiovascular effects. *Cardiovasc. Res.* 49: 27-37.

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