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PRODUCT DATASHEET

ChemiScreen™ VPAC1 VIP and PACAP Receptor Stable Cell Line

CATALOG NUMBER: HTS043C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

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). The 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 antagonists of interactions between $VPAC_1$ and its ligands.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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 Fluorescence Assay, cAMP accumulation

APPLICATION DATA

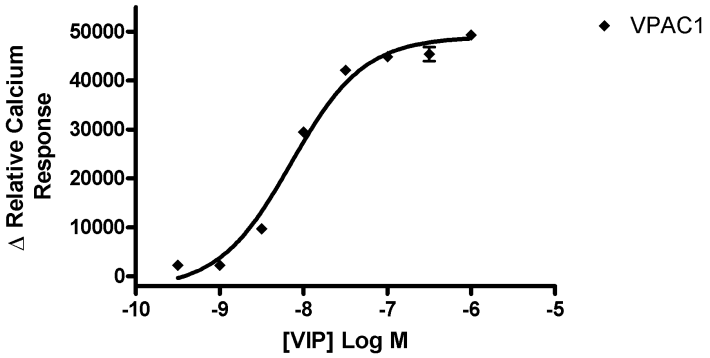

Figure 1. Representative data for activation of the $VPAC_1$ receptor stably expressed in Chem-1 cells induced by VIP using a fluorescent calcium flux assay. $VPAC_1$–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 7,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

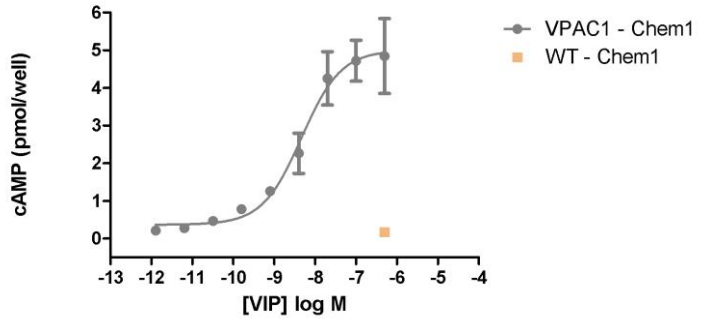

Figure 2. Representative data for activation of $VPAC_1$ receptor stably expressed in Chem-1 cells induced by VIP using a cAMP accumulation assay. $VPAC_1$–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were treated with VIP for 10 minutes in the presence of 100 µM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation OR [with Ligand for 10 minutes in the presence of 100 µM IBMX and 10 µM forskolin, to stimulate adenylate cyclase, and 0.5% DMSO to determine the receptor's ability to inhibit cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal fluorescence signal obtained in this experiment was 6,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of $VPAC_1$-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
VIP	Calcium Flux - Fluorescence	27	Eurofins Internal Data
VIP	cAMP accumulation	5	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP

Fluorescence

Table 4. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8^{TM}, AM	AAT Bioquest: 21080
VIP ligand	Sigma: V6130
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve 5x10^{5}cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human $VPAC_1$ cDNA (Accession Number: NM_004624; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

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-1	ChemiScreen™ Chem-1 Parental Cell Line (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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