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READY-TO-ASSAY CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT VPAC$_1$ VIP/PACAP FAMILY RECEPTOR

CATALOG NUMBER:	HTS043F	**QUANTITY:**	1 vial, 1 mL
LOT NUMBER:		**CONCENTRATION:**	1 x 10^7 viable cells/mL

BACKGROUND:

Millipore's Ready-To-Assay Calcium-Optimized Cells are GPCR-expressing cell lines that are designed for simple, rapid calcium assays with no requirement for culturing cells. The user simply thaws the cells with maximal viability, dispenses into assay plates, and assays for calcium response the next day.

The Ready-To-Assay cells are derived from ChemiScreen™ calcium-optimized stable cell lines, which express the GPCR target of interest at high levels on the cell surface, in a host cell line containing high levels of the promiscuous Gα15 protein to couple the receptor to the calcium signaling pathway. The Ready-To-Assay cells are prepared by chemical treatment at a concentration optimized for effective growth arrest while maintaining high viability (>80%) after thawing and overnight plating. Pharmacological functionality of the Ready-To-Assay cells is identical to that of the originating GPCR cell line.

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). Millipore's cloned human VPAC1-expressing cell line is made in the Chem-1 host, an adherent cell line that supports high levels of recombinant VPAC1 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. The untreated VPAC1-Chem-1 cell line and the Ready-To-Assay VPAC1 cells have equivalent EC50s for VIP.

APPLICATIONS:

Calcium flux assay

SPECIFICATIONS:

	EC50 for VIP (nM)	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	2.9	5755	0.59
Continuous Passage Cells	3.8	7851	0.89

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

TRANSFECTION: Full-length human human VIPR1 cDNA encoding VPAC1 (Accession Number: L13288)

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PLATING MEDIA:
DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
100 U/mL Pen-Strep (from 100x stock, Millipore TMS-AB2-C)

PRESENTATION:

Cells are frozen at 1 x 10^7 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO.

STORAGE:

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years.

ASSAY PROTOCOL:

1) 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.
2) Transfer contents of the vial to a sterile 15 mL conical tube. Add 10 mL prewarmed plating media to the cells and mix gently to resuspend cells. Centrifuge at 200 x g. Remove all but 0.5 mL media.
3) Resuspend cells to 0.5 x 10^6 cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200 µL per well to obtain a density of approximately 1 x 10^5 cells/well.
4) Place the assay plate in a humidified 37°C incubator with 5% CO_2.
5) The cells may be assayed 16-24 hours after plating. It is recommended to wash the cells with assay buffer at least once prior to addition of loading dye.

REFERENCES:

Delgado M *et al.* (2004) The significance of vasoactive intestinal peptide in immunomodulation. *Pharmacol. Rev.* 56: 249-290.

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.

Henning RJ and Sawmiller DR (2001) Vasoactive intestinal peptide: cardiovascular effects. *Cardiovasc. Res.* 49: 27-37.

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READY-TO-ASSAY CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT $VPAC_1$ VIP/PACAP FAMILY RECEPTOR
Product No. HTS043F

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