eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ V_2 Vasopressin Receptor Frozen Cells

CATALOG NUMBER: HTS060RTA

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.

Arginine vasopressin (AVP) is a 9 amino acid peptide that functions as an antidiuretic, vasoconstrictor and neurotransmitter. The three vasopressin receptors, V_{1A}, V_{1B} and V_2, are GPCRs; V_{1A} and V_{1B} couple to Gq and calcium release, whereas V_2 couples to Gs. V_2 expressed in renal collecting ducts plays an important role in regulating renal free water excretion (Birnbaumer, 2000). Mutations in V_2 result in X-linked nephrogenic diabetes insipidus, a syndrome in which the kidney is unable to concentrate urine, leading to dehydration and hypernatremia (Birnbaumer, 2001). Conversely, elevated levels of AVP lead to hyponatremia in the syndrome of inappropriate antidiuretic hormone secretion (SIADH), congestive heart failure or cirrhosis, and V_2 selective antagonists have been developed to treat these conditions (Verbalis, 2002). Millipore's cloned human V_2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant V_2 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 V_2 and its ligands.

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 Fluorescent Assays, cAMP Accumulation Assays

APPLICATION DATA

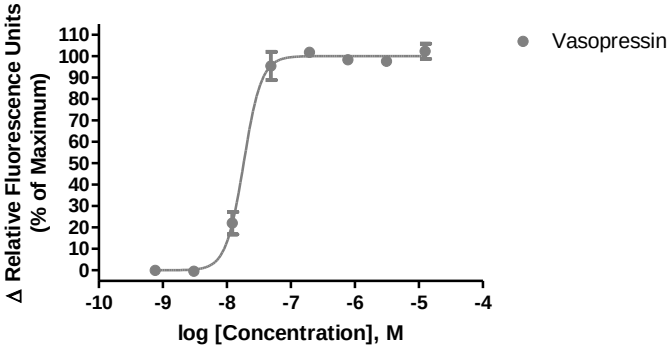

Figure 1. Representative data for activation of V_2 receptor. Calcium flux in V_2–expressing Chem-1 cell line induced by Vasopressin. V_2–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 4,500 RLU (Relative Light Units).

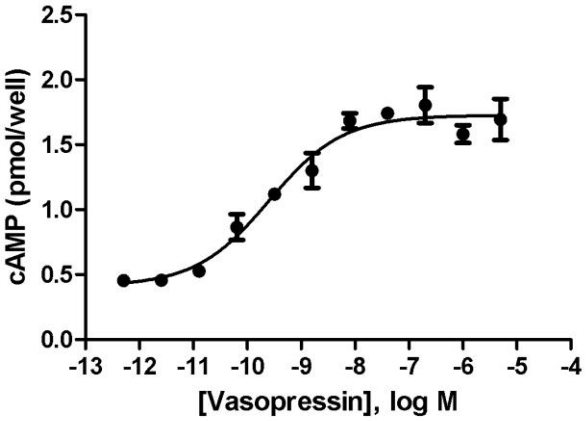

Figure 1. Representative data for activation of V_2receptor stably expressed in CHEM-1 cells induced by Vasopressin using a cAMP accumulation assay. V_2-expressing CHEM-1 cells were seeded at 100,000 cells per well into a 96-well plate, and the following day the cells were treated with Dopamine for 15 minutes in the presence of 2.0 mM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal cAMP response obtained in this experiment was 2 pmol/well. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. Comparison of EC_{50} values of V_2 Vasopressin Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Vasopressin	Calcium Flux	18	Eurofins Internal Data
Vasopressin	cAMP Accumulation	0.25	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
Vasopressin ligand	Sigma: V9879
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

V_2 cDNA (Accession Number: NM_000054; see CODING SEQUENCE below) expressed from a proprietary E5 promoter plasmid.

CODING SEQUENCE

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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS160M	ChemiScreen™ V_2 Vasopressin receptor membrane prep

REFERENCES

1. Birnbaumer M (2000) Vasopressin receptors. Trends Endocrinol. Metab. 11:406-10.
2. Birnbaumer M (2001) The V2 vasopressin receptor mutations and fluid homeostasis. Cardiovasc. Res. 51: 409-415.
3. Verbalis JG (2002) Vasopressin V2 receptor antagonists. J. Mol. Endocrinol. 29: 1-9.

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