

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT V_{1B} VASOPRESSIN RECEPTOR**

CATALOG NUMBER:	HTS136C	QUANTITY:	2 vials, 1 mL per vial
LOT NUMBER:		CONCENTRATION:	2 x 10 ⁶ cells/mL

BACKGROUND:

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 V₂, are GPCRs; V_{1A} and V_{1B} couple to G_q and calcium release, whereas V₂ couples to G_s (Birnbauer, 2000). The V_{1B} receptor is expressed prominently in the anterior pituitary, where it mediates vasopressin-induced release of ACTH (Tanoue *et al.*, 2004). A selective antagonist of V_{1B} has recently been developed and shown to reduce depression, anxiety, and aggression in rodents (Blanchard *et al.*, 2005; Griebel *et al.*, 2002). Millipore's cloned human V_{1B}-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant V_{1B} expression on the cell surface and contains high levels of the promiscuous G protein G α 15 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_{1B} and its ligands.

APPLICATIONS:

Calcium flux assay, ligand binding assays

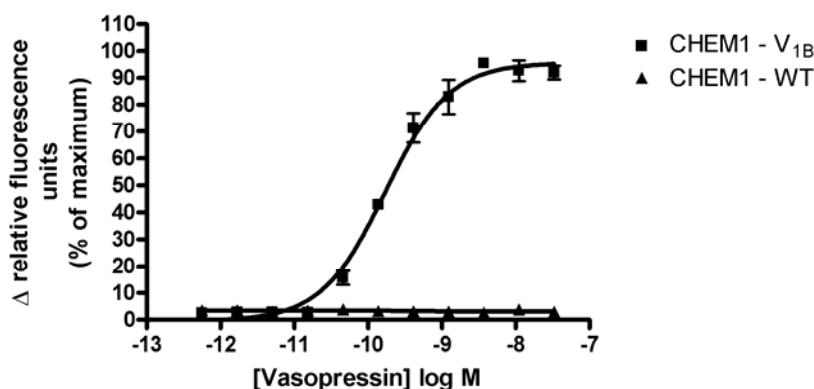


Figure 1. Calcium flux in V_{1B}-expressing Chem-1 cell line induced by arginine vasopressin. V_{1B}-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to arginine vasopressin (10⁻⁵ to 10⁻⁹ M) was determined in triplicate on a Molecular Devices FLIPR^{TETRA™}.

SPECIFICATIONS: EC₅₀ for calcium mobilization by arginine vasopressin: ~ 0.17 nM
Z' = 0.72 with arginine vasopressin at 2x EC₅₀

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, G α 15.

TRANSFECTION: Full-length human AVPR1B cDNA encoding V_{1B} (Accession Number:

NM_000707)

PRESENTATION:

Cells are frozen at 2×10^6 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.

STORAGE/HANDLING:

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37°C water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol. Transfer contents of the vial to a T75 flask containing growth media. Place the flask in a humidified incubator at 37°C with 5% CO₂.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca⁺⁺ and Mg⁺⁺ (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37°C with 5% CO₂ until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem-1 Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at 5×10^6 cells/mL in Chem-1 Freezing Media (cell densities of $2-10 \times 10^6$ are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at -70°C overnight. Store the vials in liquid nitrogen.
7. Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays. Cells should be resuspended in Chem-1 Plating Media for plating for calcium assay.

MEDIA:

Chem-1 Growth 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)
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)
250µg/mL Genetecin/G-418

Chem-1 Plating Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine
10% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep

Chem-1 Freezing Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine
20% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep
10% DMSO (cell culture grade)

REFERENCES:

- Birnbaumer M (2000) Vasopressin receptors. *Trends Endocrinol. Metab.* 11:406-10.
- Blanchard RJ *et al.* (2005) AVP V1b selective antagonist SSR149415 blocks aggressive behaviors in hamsters. *Pharmacol. Biochem. Behav.* 80: 189-194.
- Griebel G *et al.* (2002) Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V_{1b} receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. *Proc. Natl. Acad. Sci. USA* 99: 6370-6375.
- Tanoue A *et al.* (2004) The vasopressin V1b receptor critically regulates hypothalamic-pituitary-adrenal axis activity under both stress and resting conditions. *J. Clin. Invest.* 113: 302-309.

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HUMAN RECOMBINANT V_{1B} VASOPRESSIN RECEPTOR****Product No. HTS136C**

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