Human V1B Receptor Membrane Preparation

Technical Manual No. TM0497 **Version 10132010**

I. Introduction

Catalog Number: M00401

Cell Line: CHO-K1/V1B

Expressed Gene: GenBank Accession Number NM_000706; no expressed tags

Alternate Names: V1BR, arginine vasopressin receptor 1B

Host Cell: CHO-K1

Quantity: 1 vial (1 ml per vial)

Protein Concentration: 2 mg/ml

Buffer: 50 mM HEPES, 5 mM $MgCl_2$, 1 mM $CaCl_2$ and 0.2% BSA, pH7.4

Application: Binding assay for V1B receptor

Storage: Store at -80°C

II. Background:

The antidiuretic hormone arginine vasopressin (AVP) receptors are G protein-coupled receptors which consists of at least three types: V1A (vascular/hepatic) and V1B (anterior pituitary) receptors, which act through phosphatidylinositol hydrolysis to mobilize intracellular Ca^{2+}, and V2 (kidney) receptor, which is coupled to adenylate cyclase. V1B receptors are expressed in anterior pituitary where they mediate the release of ACTH. Its peripheral actions, such as antidiuresis, contraction of vascular smooth muscle, and stimulation of hepatic glycogenolysis are well characterized.

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III. Representative Data

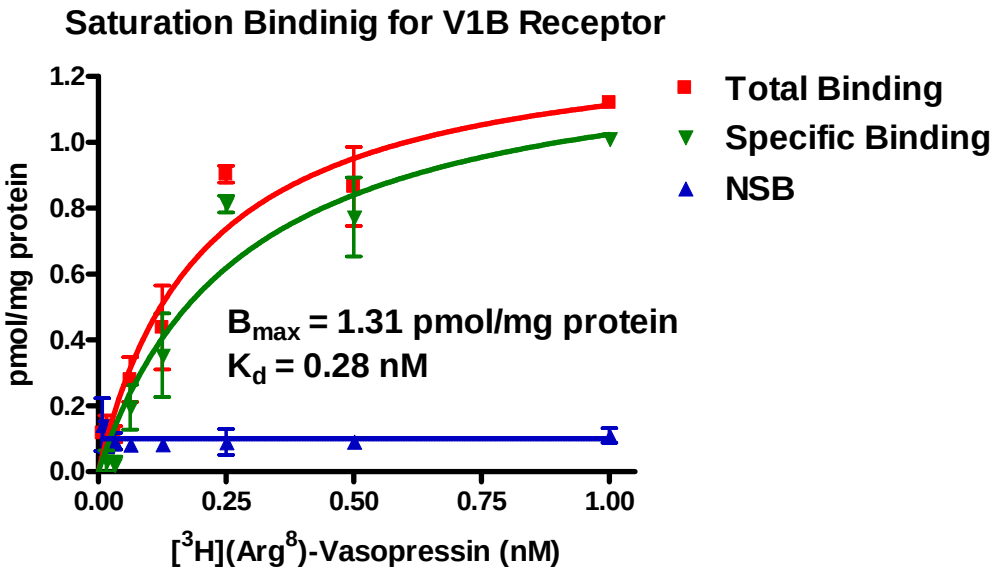

Figure 1 10 µg of membranes prepared from CHO-K1 cells stably expressing V1B receptors were incubated with indicated concentrations of [^{3}H](Arg^8)Vasopressin in the absence (total binding) or presence of 1000-fold excess unlabeled (Arg^8)Vasopressin (nonspecific binding, NSB). Binding was terminated by rapid filtration. Specific binding was defined by subtracting NSB from total binding. Data were fit to one-site binding equation using a non-linear regression method.

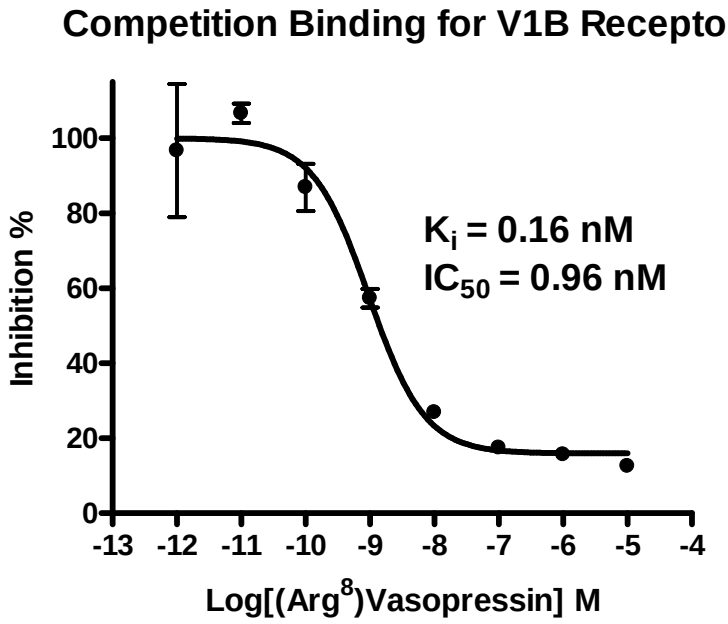

Figure 2 20 µg of membranes prepared from CHO-K1 cells stably expressing V1B receptors were incubated with indicated concentrations of (Arg^8)Vasopressin in the presence of 1.4 nM [^{3}H](Arg^8)Vasopressin. Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear regression method.

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IV. Brief Competition Binding Protocol

1. Incubated 20 μg membranes with radio labeled ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate. Incubated for 60 min at 37°C.
2. Prior to filtration, coat a GF/C 96-well filter plate with 0.5% polyethyleneimine (PEI) for 30 min at 4°C, then washed the plate with 1 ml/well 50mM HEPES, 0.5% BSA (pH 7.4).
3. Transfer the binding mixtures then to the filter plate. After quick filtration, wash the plate for 3 times (3 ml per well totally) with Wash Buffer.
4. Dry the plate for 0.5 h and then add 50 μl scintillation cocktail (Microscint20). Stay for 1min and count on TopCount NXT for 1 min/well.
5. Binding buffer: 50 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C
6. Wash Buffer: 50 mM HEPES, pH 7.4, 500 mM NaCl, 0.1% BSA, filtered and stored at 4°C.

V. References

1. Sugimoto T *et al*. (1994) Molecular cloning and functional expression of a cDNA encoding the human V1b vasopressin receptor. *J Biol Chem*. 269(43):27088-92.
2. Tahara A, *et al*. (1998) Pharmacological characterization of the human vasopressin receptor subtypes stably expressed in Chinese hamster ovary cells. *Br J Pharmacol.* 125(7):1463-70.
3. Serradeil-Le Gal C *et al*. (2007) Biological characterization of rodent and human vasopressin V1b receptors using SSR-149415, a nonpeptide V1b receptor ligand. *Am J Physiol Regul Integr Comp Physiol.* 293(2):R938-49. Epub 2007 May 23.

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