Human Recombinant ADRA1A Adrenoceptors Stable Cell Line

Technical Manual No. TM0429 **Version 10132010**

I. Introduction

Catalog Number: M00225

Cell Line Name: CHO-K1/ADRA1A

Gene Synonyms: α_1 -adrenergic receptor, ADRA1A

Expressed Gene: Genbank Accession Number NM_000680; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for ADRA1A receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 400 µg/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

The α_1-adrenergic receptor (AR) family consists of three closely related gene products (α_{1A}, α_{1B}, and α_{1D}) that mediate the actions of norepinephrine (NE) and epinephrine in sympathetically innervated tissues and brain. α_1-ARs belong to the G protein-coupled receptor family and consist of single polypeptide chains predicted to have seven transmembrane spanning domains. With similar pharmacological and signaling properties, α_1-AR subtypes act through Gq/11 proteins to activate phospholipase C, increase both inositol 1,4,5-trisphosphate production and intracellular Ca^{2+}. Once activated by binding, α_1-ARs initiate the cellular pathways leading to the regulation of physiological effects, including blood pressure maintenance, glucose metabolism, renal sodium reabsorption, and cardiac inotropy.

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III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by Epinephrine in CHO-K1/ADRA1A and CHO-K1 cells

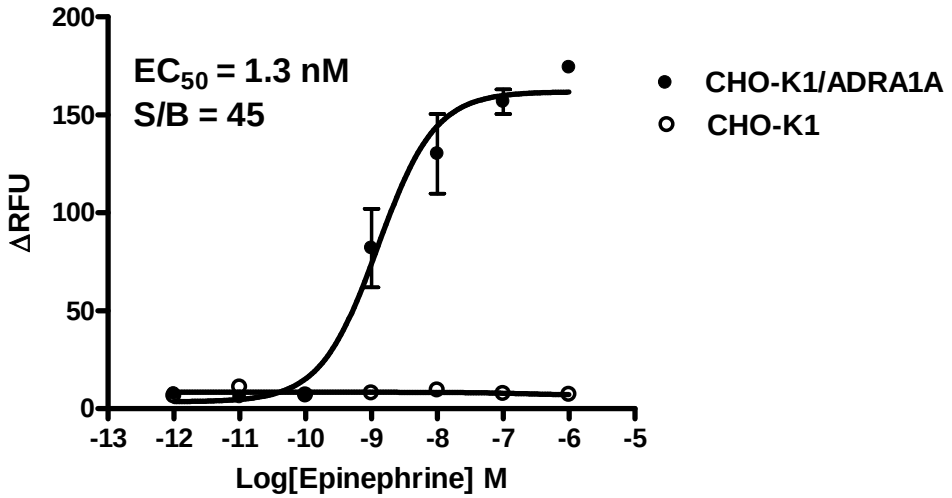

Figure 1. Epinephrine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADRA1A and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an ADRA1A receptor agonist, Epinephrine. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Epinephrine (Mean ± SD, n = 2). The EC_{50} of Epinephrine on ADRA1A in CHO-K1 cells was 1.3 nM. The S/B of Epinephrine on ADRA1A in CHO-K1 cells was 45.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:

   Y=Bottom + (Top-Bottom)/(1+10^((LogEC_{50}-X)*HillSlope))

   X is the logarithm of concentration. Y is the response

   Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.

2. Signal to background Ratio (S/B) = Top/Bottom

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Radioligand Binding Assay

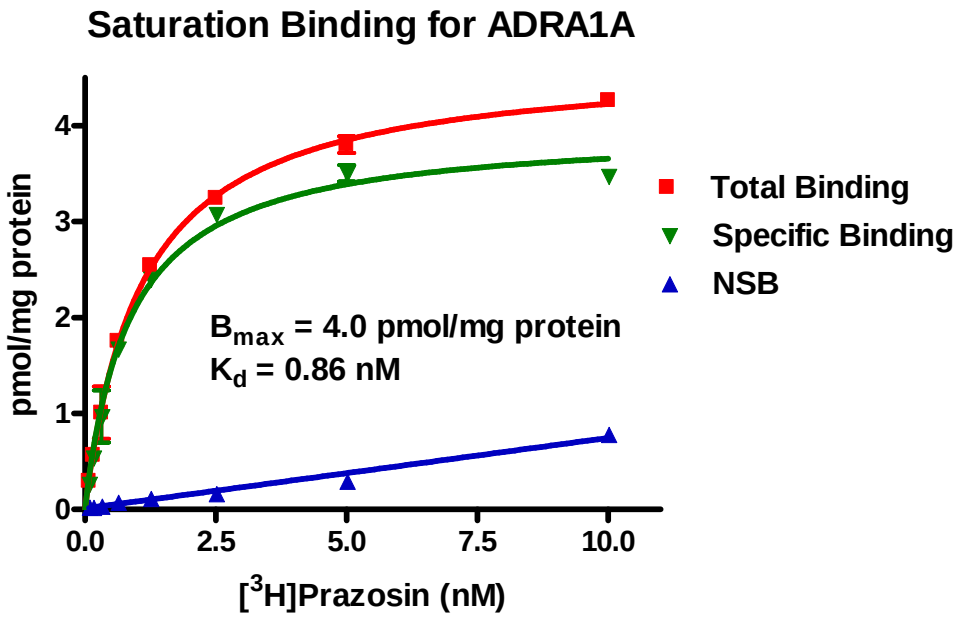

Figure 2 10 µg of membranes prepared from CHO cells stably expressing ADRA1A receptors were incubated with indicated concentrations of [^{3}H]Prazosin in the absence (total binding) or presence of 1000-fold excess unlabeled Prazosin (nonspecific binding, NSB). Binding was terminated by rapid filtration. Specific binding was defined by subtracting NSB from total binding. Data were fit to one-site binding equation using a non-linear regression method.

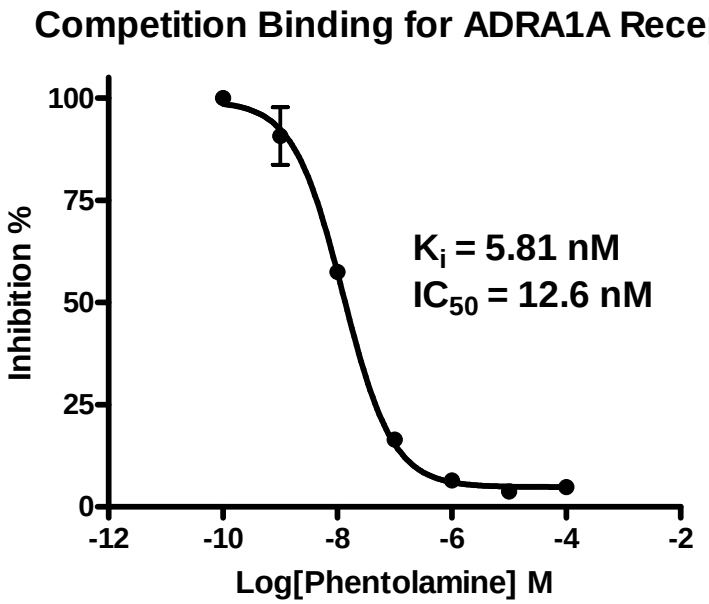

Figure 3 10 µg of membranes prepared from CHO cells stably expressing ADRA1A receptors were incubated with indicated concentrations of Phentolamine in the presence of 1nM [^{3}H]Prazosin. Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear regression method.

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IV. Thawing and Subculturing

Thawing: Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g force for 5 min, and discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add G418 to a concentration of 400 µg/ml the following day.

Subculturing: Protocol

1. Remove and discard culture medium.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution to 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
   Note: To avoid clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. References

1. Vicentic, A., Robeva, A., Rogge, G., Uberti, M. and Minneman, K.P.(2002) Biochemistry and Pharmacology of Epitope-Tagged α_1--Adrenergic Receptor Subtypes *J. Pharmacol. Exp. Ther., 302: 58-65*
2. Zhong H and Minneman KP (1999a) α_1-Adrenoceptor subtypes. *Eur J Pharmacol 375:261–276.*
3. Ruffolo RR Jr, Stadel JM, and Hieble JP (1994) α1-Adrenoceptors: recent developments. *Med Res Rev 14:229–270.*
4. Esbenshade TA, Han C, Murphy TJ, and Minneman KP (1993) Comparison ofα_1--adrenergic receptor subtypes and signal transduction in SK-N-MC and NB41A3 neuronal cell lines. *Mol Pharmacol 44:76–86.*

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