Human Recombinant ADRA1A Adrenoceptors Stable Cell Line
Cat. No. M00225 **Version 07272020**

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
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for ADRA1A receptor (calcium flux assay, IP-One assay)
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F12K (Kaighn's) (Cat. #21127-022, Gibco), 10% FBS
Culture Medium: Ham's F12 K (Kaighn's), 10% FBS, 400 µg/ml G418 (Cat. #10131-035, Gibco)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

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_1ARs 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.

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.*

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Epinephrine in CHOK1/ADRA1A cells

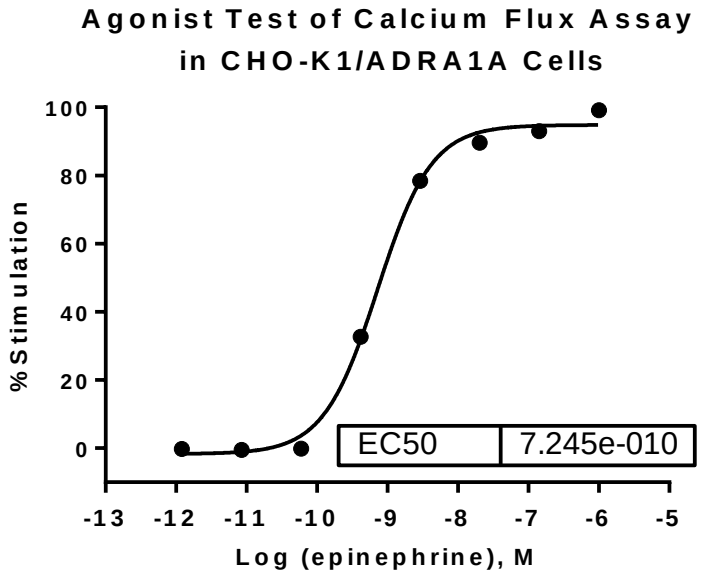

Figure 1: Epinephrine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHOK1/ADRA1A cells. The cells were loaded with Calcium-4 prior to being stimulated with an ADRA1A receptor agonist, epinephrine. The intracellular calcium change was measured by FLIPR. The relative 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 this cell was 0.72 nM.

Note:

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.

Radioligand Binding Assay

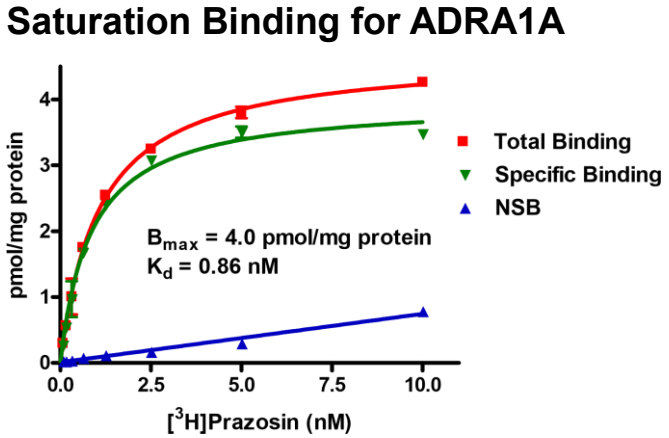

Figure 2: 10 µg of membranes prepared from CHO cells stably expressing ADRA1A receptors were incubated with indicated concentrations of [^{3}H]Prazosin in the absence (total binding) or presence of 1000-fold excess unlabeled Prazosin (nonspecific binding, NSB). Binding was terminated by rapid filtration.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

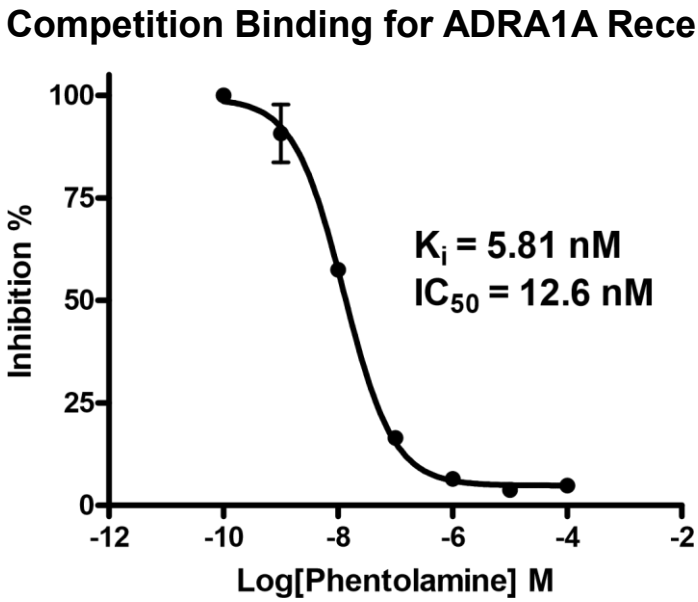

Figure 3: 10 µg of membranes prepared from CHO cells stably expressing ADRA1A receptors were incubated with indicated concentrations of Phentolamine in the presence of 1 nM [^{3}H]Prazosin. Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear regression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

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.

Sub-culturing 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).
4. 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.
5. 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.
6. Resuspend the cells in culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37°C.

Subcultivation Ratio: 1:3 to 1:8
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 α1--Adrenergic Receptor Subtypes. *J. Pharmacol. Exp. Ther*., 302: 58-65
2. Zhong H and Minneman KP (1999a) α1-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 ofα1--adrenergic receptor subtypes and signal transduction in SK-N-MC and NB41A3 neuronal cell lines. *Mol Pharmacol* 44:76–86.

GenScript USA Inc,
860 Centennial Ave.
Piscataway, NJ 08854
Toll-Free: 1-877-436-7274
Tel: 1-732-885-9188, Fax: 1-732-210-0262
Email: product@genscript.com
Web: http://www.genscript.com
For Research Use Only.

Limited Use License Agreement

This is a legal agreement between you (Licensee) and GenScript USA Inc. governing use of GenScript's stable cell line products and protocols provided to licensee. By purchasing and using the stable cell line, the buyer agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:

1) The products are not transferable and will be used at the site where they were purchased. Transfer to another site owned by buyer will be permitted only upon written request by buyer followed by subsequent written approval by GenScript.
2) The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party.
3) The products sold by GenScript are for laboratory and animal research purposes only. The products are not to be used on humans, for consumption, or for any unlawful uses.

GenScript USA Inc. will not assert against the buyer a claim of infringement of patents owned or controlled by GenScript USA Inc. and claiming this product based upon the manufacture, use or sale of a clinical diagnostic, therapeutic and vaccine, or prophylactic product developed in research by the buyer in which this product or its components has been employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on the use of this product for other purposes, contact Marketing Department, GenScript USA Inc., 860 Centennial Ave. Piscataway, NJ 08854, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.