Human Recombinant D1 Dopamine Receptor Stable Cell Line
Cat. No. M00247 **Version 07272020**

I. INTRODUCTION

Catalog Number: M00247
Cell Line Name: CHO-K1/D1
Gene Synonyms: DRD1; DADR; DRD1A
Expressed Gene: Genbank Accession Number NM_000794; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for D1 receptor
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F-12 K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Culture Medium: Ham's F-12 K (Kaighn's), 10% FBS, 200 µg/ml Zeocin (Cat. #R250-01, Life Technologoes)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

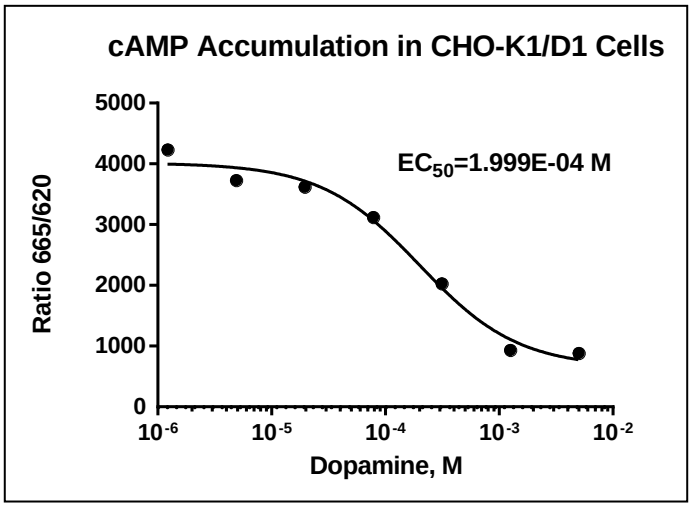
II. BACKGROUND

Dopamine is the predominant catecholamine neurotransmitter found in mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation. It also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function, and gastrointestinal motility. The dopamine receptor family consists of five members, which are classified into two groups, D1-like (D1 and D5) and D2-like (D2, D3, and D4).

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

III. ASSAY DEVELOPMENT

This cell-based assay is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). It is a competitive immunoassay that uses cAMP labeled with the d2 acceptor flourophore and an anti-cAMP monoclonal AB labeled with Europium Cryptate. The FRET signal decreases as cAMP concentration rises.



Agonist Assay Protocol

1. Seed 5 µl CHO-K1/D1 cells into a 384-well low volume plate, 3,000 cells per well.
2. Add 5 µl compound or dopamine (diluted in buffer) to each well and incubate the plate for 30 min at 23°C.
3. Add 5ul of cAMP-d2 conjugate solution to each well.
4. Add 5µl of cAMP-AB lysis buffer solution to each well.
5. Incubate the plate in the dark for one hour at 23°C.
6. Read the plate PHERAstar PLUS (BMG Labtech, Offenburg, Germany).

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 remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %CO_2.
7. Add antibiotic in the following day.

Sub-culturing Protocol

1. Remove the culture medium from cells.
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 into 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 cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.

4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO_2.

Subcultivation Ratio: 1:3 to 1:8

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Bergson, C, *et al*. (1995) Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *J Neurosci*. 12:7821-36
2. Dearry, A, *et al*. (1990) Molecular cloning and expression of the gene for a human D1 dopamine receptor. *Nature* 347:72-76
3. Missale C, (1998) Dopamine receptors: from structure to function. *Physiol Rev*. 78(1):189-225.

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.