Human Recombinant CHO-K1/ACE2 Stable Cell Line
Cat. No. M00771 **Version 04242020**

I. INTRODUCTION

Catalog Number: M00771
Cell Line Name: CHO-K1/ACE2
Gene Synonyms: Angiotensin-converting enzyme 2, ACEH
Expressed Gene: Codon Optimized from NM_021804.2; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (>1×10^6 cells/vial)
Stability: 15 passages
Application: Binding assay or use as immunogen
Freeze Medium: 95% Culture Medium, 5% (V/V) DMSO
Complete Growth Medium: F12K, 10% FBS
Culture Medium: F12K, 10% FBS, 250μg/ml Hygromycin
Mycoplasma Status: Not detected*
Storage: Liquid nitrogen immediately upon receipt

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

II. BACKGROUND

Angiotensin-converting enzyme 2 (ACE2), discovered as a homologue of ACE, acts as its physiological counterbalance providing homeostatic regulation of circulating angiotensin II (Ang II) levels. ACE2 is a zinc metalloenzyme and carboxypeptidase located as an ectoenzyme on the surface of endothelial and other cells. While its primary substrate appears to be Ang II, it can hydrolyze a number of other physiological substrates. Additionally, ACE2 functions in other noncatalytic cellular roles including the regulation of intestinal neutral amino acid transport. It also

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serendipitously acts as the receptor for the severe acute respiratory syndrome virus. Upregulation of ACE2 expression and function is increasingly recognized as a potential therapeutic strategy in hypertension and cardiovascular disease, diabetes, lung injury, and fibrotic disorders. ACE2 is regulated at multiple levels including transcriptional, posttranscriptional (miRNA and epigenetic), and posttranslational through its shedding from the cell surface.

III. REPRESENTATIVE DATA

Protein Expression Validation

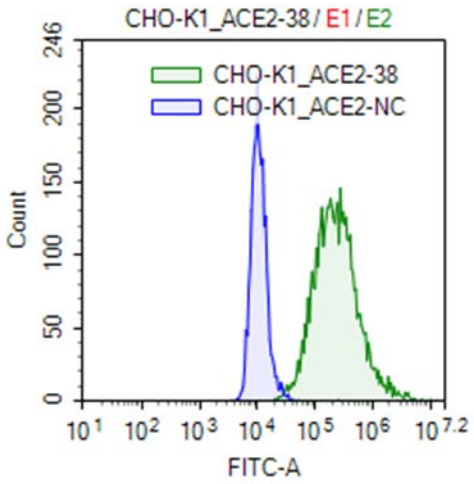

Figure 1. FACS analysis of ACE2 expression in CHO-K1/ACE2 clone 38.

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 800 rpm for 4 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 the following day.

Sub-culturing Protocol

1. Remove the culture medium from cells.

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2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.25% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25200-072) 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 800 rpm for 4 min, 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: 1:4 to 1:8
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Anthony J. Turner. *The Protective Arm of the Renin Angiotensin System (RAS)*, 2015
2. Stertz S. *The intracellular sites of early replication and budding of SARS-coronavirus*, 2007

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