Version: 01
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Cynomolgus Recombinant CD155 Stable Cell Line
Cat. No. M00643

I. INTRODUCTION

Recombinant CHO-K1 cells stably overexpress *Macaca fascicularis* PVR cell adhesion molecule (CD155) on the surface. The surface expression of CD155 is validated by FACS analysis. This cell line is recommended for cell-based binding assay to screen antibodies against cyno CD155 or to measure binding affinity between CD155 and anti-CD155 antibodies.

Catalog Number: M00643
Cell Line Name: CHO-K1/cyno CD155
Gene Synonyms: PVR, HVED, NECL5, Necl-5, PVS, TAGE4
Expressed Gene: NCBI reference sequence NM_001043386.1; no expressed tags
Target Protein: NP_001036851.1
Host Cell: CHO-K1
Size: Two vials of frozen cells (>1×10^6 per vial in 1 mL)
Culture Properties: Adherent
Freeze Medium: 95% complete growth medium, 5% (V/V) DMSO (Cat. No. D2650, Sigma)
Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. No. 21127-022, Life Technologies), 10% FBS (Cat. No. 10099-141, Life Technologies)
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 8 μg/ml Puromycin (Cat. No. A11138-03, Life Technologies)
Stability: Stable through more than 16 passages without significant changes in assay performance or expression profile.
Application: Binding assay or use as immunogen
Mycoplasma Status: Negative. The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit (Cat. No. LT07-318, Lonza).
Storage: Store cells in liquid nitrogen immediately upon receipt. Thaw and recover cells within

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one year from the date received.

II. BACKGROUND

CD155, commonly known as PVR (poliovirus receptor) and Necl-5 (nectin-like molecule-5), is a type I transmembrane single-span glycoprotein, and belongs to the nectins and nectin-like (Necl) subfamily. CD155 was originally identified based on its ability to mediate the cell attachment and entry of poliovirus (PV), an etiologic agent of the central nervous system disease poliomyelitis. The normal cellular function is in the establishment of intercellular adherent junctions between epithelial cells. CD155 may assist in an efficient humoral immune response generated within the intestinal immune system. It has been demonstrated that CD155 can be recognized and bond by DNAM-1 and CD96 which promote the adhension, migration and NK-cell killing, and thus efficiently prime cell-mediated tumor-specific immunity.

III. REPRESENTATIVE DATA

FACS Analysis

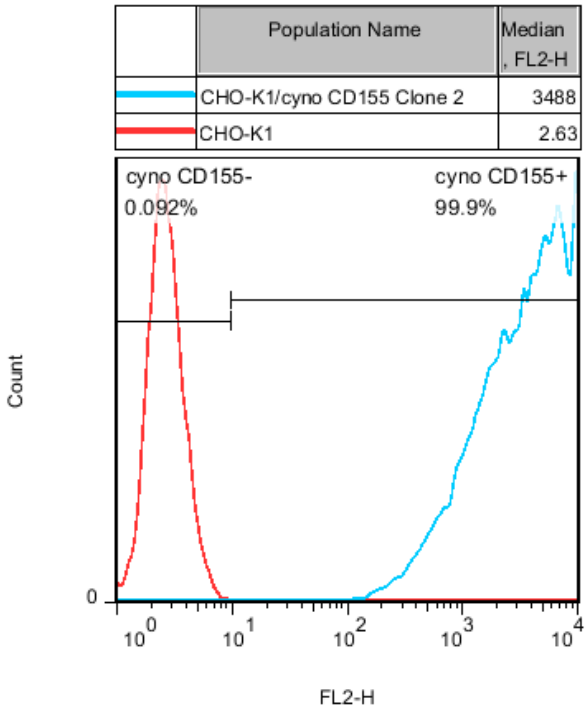

Figure 1. FACS analysis of cell surface expression of cynomolgus CD155 on CHO-K1/cyno CD155 cells. The CHO-K1/cyno CD155 cells (blue) and the negative control CHO-K1 cells (red) were probed using PE-conjugated anti-human CD155 antibody (Cat. No. 337610, Biolegend).

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IV. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial containing the frozen cells from liquid nitrogen tank and place into a 37°C water bath immediately.
2. Thaw the cells quickly (within 1-2 minutes) by gently swirling the vial. Do not vortex the cells.
3. When the cells are almost completely thawed, take the vial out of the water bath and decontaminate it with 70% ethanol.
4. In a biosafety hood, transfer the cells to a sterile 15 ml conical tube. Add 9 ml of complete growth medium to the cells.
5. Pellet cells by centrifugation at 200 × g for 3-5 minutes at room temperature.
6. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure that the cell pellet is not disturbed.
7. Resuspend the cells by gently flicking the tube. Gently add in 10 ml of complete growth medium.
8. Transfer the cell suspension into a 10 cm culture dish containing 10 ml of complete growth medium.
9. Grow the cells in an incubator at 37°C with 5% CO_2.
10. The cells will attach the dish in about 2-4 days. Check the status of the cells every day and don't disturb the cells till most cells attach well.
11. Change the medium with culture medium when cells grow well.

Sub-culturing Protocol

1. Remove the culture medium from the cells.
2. Wash cells with sterile PBS to remove all traces of serum which contains trypsin inhibitors.
3. Add 0.25% Trypsin/EDTA (Cat. No. 25200, Gibco) solution to the culture dish and observe the cells under an inverted microscope until the cell layer has dispersed (usually within 3-5 minutes).
   Notes: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. If cells are difficult to detach, place the dish in a 37°C incubator for about 2 minutes.
4. Add 6-8 ml of complete growth medium to the culture dish, aspirate the medium with cells by gentle pipetting and then add into a sterile falcon tube.
5. Centrifuge the cells at 200 x g for 5 minutes, and remove the medium.
6. Resuspend the cells in culture medium and add the cell suspension to a new culture dish.
7. Grow the cells in an incubator at 37°C with 5% CO_2.
8. The cells will attach the dish in about 4 days. Don't disturb the cells till most cells attach well.
9. Change the medium with culture medium when cells grow well.

Notes:

Subcultivation Ratio: 1:3 to 1:8.

Medium Renewal: Every 2 to 3 days.

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Cryopreservation Protocol

1. Remove the cell culture medium, wash the cells with PBS once (optional), gently add enough trypsin to cover the cells and incubate for approximately 2 minutes in a 37°C incubator.
2. Resuspend in cell culture medium and transfer into a sterile 50 ml conical tube.
3. Count the viable cells using a hemocytometer. If preferred, also determine the cell viability. Cell viability should be at least 90% for good cryopreservation.
4. Centrifuge the cells at about 200 × g for 5 minutes at room temperature to pellet cells. Remove the supernatant gently without disturbing the cell pellet.
5. Resuspend cells by adding freezing medium to the tube to the required cell density (2-5 × 10^6 cells/ml for best results).
6. Aliquot 1 ml each into cryogenic storage vials and secure the lids.
7. Transfer the vials into a cryo-freezing container at room temperature and put into a -80°C freezer. The temperature inside the cryo-freezing container should decrease steadily by 1°C/minute.
8. After approximately 24 hours, remove the vials from the cyro-freezing container and transfer into liquid nitrogen for long term storage.

V. REFERENCES

1. Freistadt MS, *et al*. Hematopoietic cells from CD155-transgenic mice express CD155 and support poliovirus replication *ex vivo* [J]. *Microb Pathog*. 2000, 29 (4): 203-212.
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3. Kakunaga S, *et al*. Enhancement of serum- and platelet-derived growth factor-induced cell proliferation by Necl-5/Tage4/poliovirus receptor/CD155 through the Ras-Raf-MEK-ERK signaling [J]. *J Biol Chem*, 2004, 279 (35): 36419-36425.
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5. Minami Y, *et al*. Involvement of up-regulated Necl-5/Tage4/PVR/CD155 in the loss of contact inhibition in transformed NIH3T3 cells [J]. *Biochem Biophys Res Commun*, 2007, 352 (4): 856-860.

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