Human Recombinant CD47 Stable Cell Line
Cat. No. M00581

Version 1

Update 12/26/2022

I. INTRODUCTION

Recombinant CHO-K1 cells stably overexpress *Homo sapiens* CD47 molecule (CD47) on the cell surface. The surface expression of CD47 is validated by FACS analysis. This cell line is recommended for cell-based binding assay to screening antibodies against CD47 or to measure binding affinity between CD47 and anti-CD47 antibodies.

Catalog Number: M00581
Cell Line Name: CHO-K1/CD47
Gene Synonyms: IAP, MER6, OA3
Expressed Gene: Codon optimized from NM_001777.3; no expressed tags
Target Protein: NP_001768.1
Host Cell: CHO-K1
Size: 2 vials of frozen cells (>1×10^6 per vial in 1 ml)
Culture Properties: Adherent
Freeze Medium: 95% complete growth medium, 5% DMSO (Cat. No.D2650, Sigma)
Complete Growth Medium: Ham's F-12K (Kaighn's) Medium (Cat. No. 21127-022, Gibco), 10% FBS (Cat. No. 10099-141, Gibco)
Culture Medium: Ham's F-12K (Kaighn's) Medium (Cat. No. 21127-022, Gibco), 10% FBS (Cat. No. 10099-141, Gibco), 4 μg/ml Puromycin (Cat. No. A11138-03, Gibco)
Stability: Stable through more than 15 passages with no significant changes in assay performance or expression profile.
Applications: Cell-based 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 one year from the date received.

II. BACKGROUND

CD47 (Cluster of Differentiation 47), also known as integrin-associated protein (IAP), is a transmembrane protein that in humans is encoded by *CD47* gene. CD47 belongs to the immunoglobulin superfamily and partners with membrane integrins and also binds the ligands thrombospondin-1 (TSP-1) and signal-regulatory protein alpha (SIRPα).

CD47 is involved in a range of cellular processes, including apoptosis, proliferation, adhesion, and migration. Furthermore, it plays a key role in immune and angiogenic responses. CD47 is ubiquitously expressed in human cells and has been found to be overexpressed in many different tumor cells.

III. REPRESENTATIVE DATA

FACS Analysis

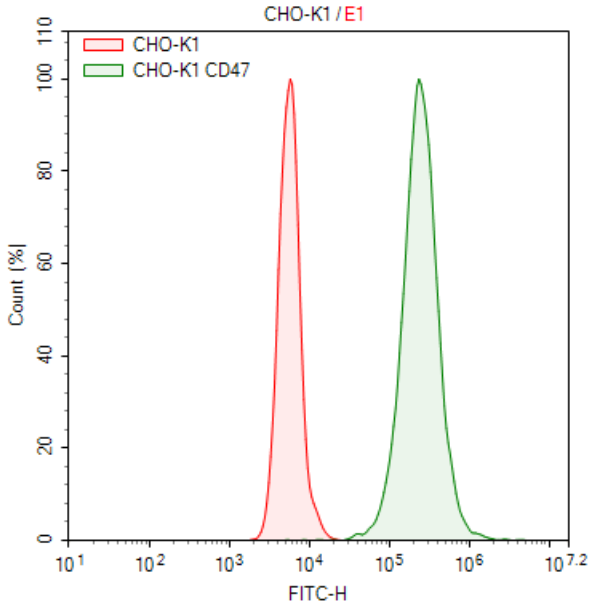

Figure 1: FACS analysis of cell surface expression of CD47 on CHO-K1/CD47 cells. The CHO-K1/CD47 cells (Green) and the negative control CHO-K1 cells (Red) were probed using Human anti-CD47 antibody (Hu5F9) (Cat. No. B3048, BIOINTRON) and Alexa Fluor™ 488 conjugated Alexa Fluor™ 488 goat anti-human IgG (H+L) (Cat. No. A11013, Invitrogen).

IV. THAWING, SUBCULTURING AND CRYOPRESERVATION

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.

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 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.

   Notes:

   Subcultivation Ratio: 1:4 to 1:8.

   Medium Renewal: Every 2 to 3 days.

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. Sick E, Jeanne A, Schneider C, Dedieu S, Takeda K, Martiny L (December 2012). "CD47 update: a multifaceted actor in the tumour microenvironment of potential therapeutic interest". *Br. J. Pharmacol.* 167 (7):1415–30.
2. Chao MP, Weissman IL, Majeti R (April 2012). "The CD47-SIRPα pathway in cancer immune evasion and potential therapeutic implications". *Curr. Opin. Immunol.* 24 (2): 225–32.
3. Caston, Stephanie; Cooper, Elizabeth; Chandramani-Shivalingappa, Prashanth; Sponseller, Brett; Hostetter Jesse; Sun, Yaxuan (July 2016). "CD47 expression in cryopreserved equine cutaneous masses and normal skin". *Journal of Veterinary Diagnostic Investigation*. 28 (4): 408–413.

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