

Version: 03

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Human Recombinant CD47 Stable Cell Line

Cat. No. M00581

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I. INTRODUCTION

Recombinant CHO-K1 cells stably overexpress human CD47 on the surface. The surface expression of CD47 on this cell 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: NCBI reference sequence NM_001777.2; no expressed tags

Target Protein: NP_001768.1

Host Cell: CHO-K1

Size: Two vials of frozen cells ($>1 \times 10^6$ 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, 4 $\mu\text{g/ml}$ 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

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 (SIRP α).

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

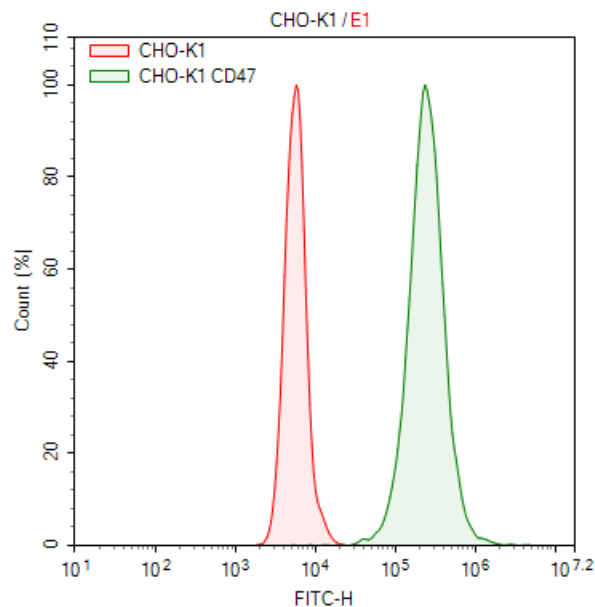


Figure 1. FACS analysis of cell surface expression of human CD47 on CHO-K1/CD47 cells. The CHO-K1/CD47 cells (Green) and the negative control CHO-K1 cells (Red) were probed using human anti-CD47 antibody (Hu5F9) (Cat. No. B3048, BIOINTRON) and Alexa Fluor™ 488 conjugated Alexa Fluor™ 488 goat anti-human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

IV. THAWING 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 $200 \times g$ 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 $200 \times g$ 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.

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 $200 \times g$ 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 ($2-5 \times 10^6$ 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 $1^{\circ}\text{C}/\text{minute}$.
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-SIRP α 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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