Human Recombinant CD47 Stable Cell Line
Cat. No. M00581 **Version 04282015**

I. INTRODUCTION

Catalog Number: M00581
Cell Line Name: CHO-K1/human CD47
Gene Synonyms: IAP, MER6, OA3
Expressed Gene: Codon Optimized from NM_001777.3; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (1×10^6 per vial)
Stability: 15 passages
Application: Binding assay or use as immunogen
Freeze Medium: 95% complete growth medium, 5% DMSO
Complete Growth Medium: F12, 10% FBS
Culture Medium: F12, 10% FBS, 4 μg/ml Puromycin
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

CD47 (Cluster of Differentiation 47) also known as integrin associated protein (IAP) is a transmembrane protein that in humans is encoded by the 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.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. neurolyticum, M. hyopneumoniae and M. capricolum) and one species Ureaplasma (U. urealyticum), with sufficient sensitivity and specificity.

III. REPRESENTATIVE DATA

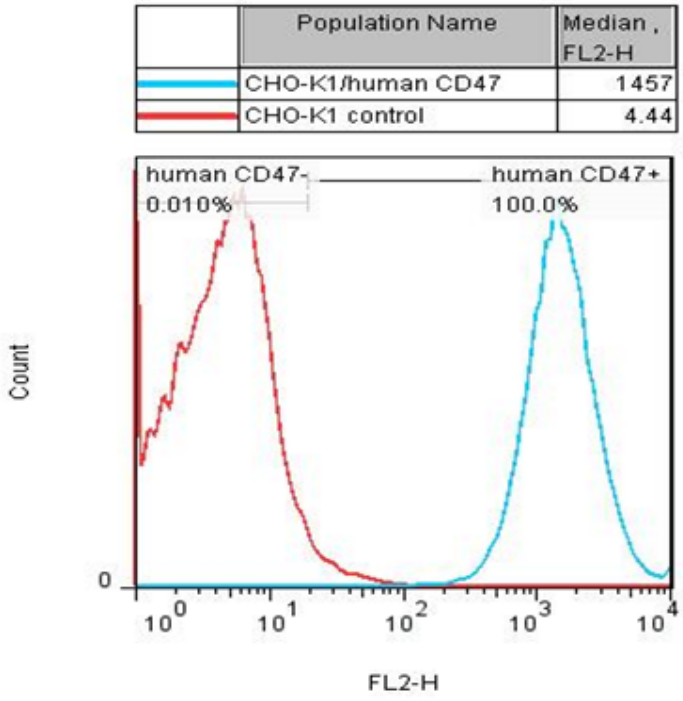

Figure 1. FACS analysis of human CD47 expression in CHO-K1/human CD47 cells.

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 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 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 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 weekly.
Medium Renewal: Every 2 to 3 days

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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