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Data Sheet
CD47 - HEK293 Cell Line
Catalog # 71249

Background
CD47 (also known as Rh-associated protein, GP42, integrin-associated protein (IAP), and neurophilin,) is an immunoglobulin-like protein that interacts with its receptor, Signal-regulatory protein alpha (SIRPα), on macrophages. This binding interaction regulates transmigration, oxidative burst cytokine production, and phagocytosis, creating a “don’t eat me” signal. CD47 is ubiquitously expressed on the surface of normal cells, but is overexpressed in numerous cancer cells, where it is thought to contribute to the resistance of tumors to phagocyte-dependent clearance.

Description
Recombinant HEK293 cell line over-expressing full length human CD47 (NM_198793.2). Surface expression is confirmed by flow cytometry.

Application
Ideal for protein binding analyses and studying SIRPα receptor activity.

Host Cell
Human Embryonic Kidney cell line (HEK293). Adherent epithelial cells.

Format
Each vial contains ~3 x 10^6 cells in 1 ml of 10% DMSO in FBS.

Storage
Store in liquid nitrogen immediately upon receipt.

Culture Medium

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Growth Medium 1F (BPS Cat. #79540): Thaw Medium 1 (BPS Cat. #60187) plus 100 µg/ml Hygromycin B (Thermo Fisher, Cat. #10687010).

Culture conditions
Frozen Cells: Prepare a 50 ml conical tube with 10 ml of pre-warmed Thaw Medium 1 (**no hygromycin)**. Quickly thaw cells in a 37°C water bath with constant and slow agitation. Clean the outside of the vial with 70% ethanol and immediately transfer the entire content to Thaw Medium 1 (**no hygromycin)**. Avoid pipetting up and down, and gently rock the conical tube.

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Spin the cells down at 150 x g for 5 min. Discard the medium and re-suspend the cell pellet in fresh Thaw Medium 1 (**no hygromycin)**. Transfer the entire content to a T25 flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2. After 48-72 hours of incubation, change to fresh Thaw Medium 1 (**no hygromycin)**, without disturbing the attached cells. Continue to change the medium every 2-3 days until the cells reach desired confluency. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture. Switch to Growth Medium 1F (**with hygromycin)** after the first passage.

Subculture: When cells reach 90% confluency, remove the medium and GENTLY wash once with PBS (without Magnesium or Calcium). These cells are loosely adherent and detach easily so do not re-suspend the PBS directly onto the cell surface. Treat cells with 2 ml of 0.25% trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 ml pre-warmed Growth Medium 1F and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes. Remove the medium and re-suspend cells in 10 ml of pre-warmed Growth Medium 1F. Dispense 5 ml of the cell suspension into a new T75 flask containing pre-warmed 20 ml Growth Medium 1F. Incubate cells in a humidified 37°C incubator with 5% CO_2. Freeze cells in freezing medium (10% DMSO in FBS) when cells reach 90% confluency. Cells have been demonstrated to be stable for at least 15 passages; BPS recommends preparing frozen stocks so cells are not used beyond passage 20.

Mycoplasma Testing
This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, Cat. #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, Cat. #LT07-518) was used as a positive control.

Application References

1. Ide K *et.al.* (2007) Role for CD47-SIRPα signaling in xenograft rejection by macrophages. *PNAS* **104:** 5062-5066.
2. Jaiswal S et.al. (2009) CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell* **138:** 271-285.

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Quality Assurance

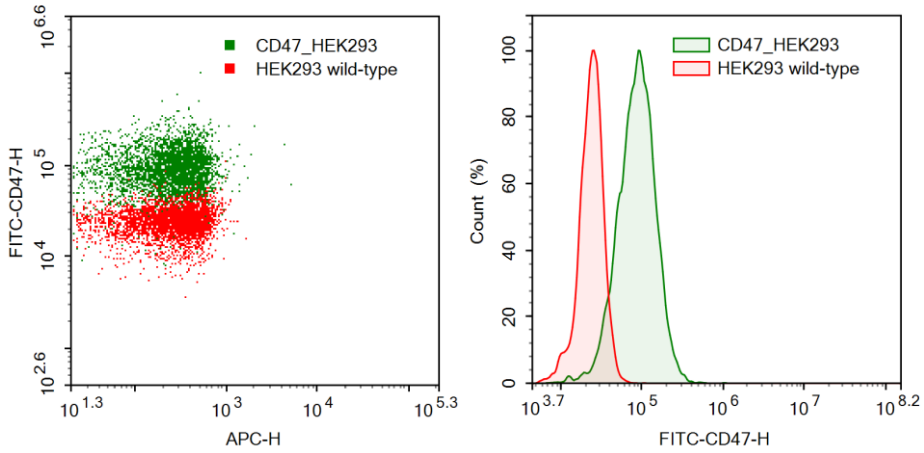

Figure 1. Expression of CD47 protein in CD47/HEK293 cells validated by Western blot and flow cytometry. Flow cytometry showed FITC conjugated anti-human CD47 antibody, clone CC2C6 (Biolegend, Cat. #323106) detects CD47-positive cells (green), using wild-type HEK293 cells as a negative control (red).

Vector and sequence

Human full length CD47 (NM_198793.2) was cloned into the MCS of pIREShyg3 vector (Clontech, Cat No. 631620).

AA Sequence (NP_942088.1)
MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFKGR
DIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELTREGETIIE
LKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIVGAILF
VPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMH
GPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRNN

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Related Products

Product	**Cat. #**	**Size**
Thaw Medium 1	60187	100 ml
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
CD47, His-Tag	71127	100 µg
CD47, Fc fusion	71177	100 µg
CD47, Fc fusion, Biotin-labeled	71169	50 µg
SIRP-α (CD172a), His-tag	71145	100 µg
SIRP-α (SIRP alpha), His-tag, Biotin-labeled	71138	50 µg
CD47:SIRP-α[Biotinylated] Inhibitor Screening Assay Kit	72044	96 rxns.
CD47:SIRP-γ[Biotinylated] Inhibitor Screening Assay Kit	72059	96 rxns.