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Data Sheet

SIRPα / HEK293 Recombinant Cell Line
Catalog # 60689

Background
SIRPα (CD172A, PTPNS1) is a member of the signal-regulatory-protein (SIRP) family, which are transmembrane immunoglobulin receptors that negatively regulate receptor tyrosine kinase-coupled signaling processes. SIRPα is expressed predominantly on macrophages and dendritic cells. Interaction with its ligand CD47 mediates signals that blocks phagocytosis (also known as the "don't eat me" signal).

Description
Recombinant HEK293 cell constitutively expressing full length human SIRPα (#NM_080792). Surface expression is confirmed by flow cytometry.

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

Format
Each vial contains ~ 2 x 10^6 cells in 1 ml of 10% DMSO in FBS.

Storage
Store in liquid nitrogen immediately upon receipt.

Application
This cell line is useful for SIRPα:CD47 protein binding analyses and screening for antibodies or inhibitors of CD47.

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

Application References
1. Lee WY *et.al*. (2010) The Role of *cis* Dimerization of Signal Regulatory Protein a (SIRPa) in Binding to CD47. *J. Biol. Chem.* **285:** 37953-37963

Culture Medium
Thaw Medium 1 (BPS Bioscience #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Thermo Fisher, Cat. #26140079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

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Growth Medium 1L (BPS Bioscience #79555): Thaw Medium 1 (BPS Bioscience, Cat. #60187) plus 2 µg/ml Puromycin Dihydrochloride (Thermo Fisher, Cat. #A1113803).

Recommended Culture Condition
Frozen Cells: Prepare a 50 ml conical tube with 10 ml of pre-warmed Thaw Medium 1 **(no puromycin)**. 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 of the vial into Thaw Medium 1 (no puromycin). Avoid pipetting up and down, and gently rock the conical tube.

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 puromycin). 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 puromycin)**, 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. Begin adding Growth Medium 1L **(contains Puromycin Dihydrochloride)** 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 medium 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. Dispense 5 ml of the cell suspension into a new T75 flask containing 20 ml pre-warmed media. 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.

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Quality Assurance

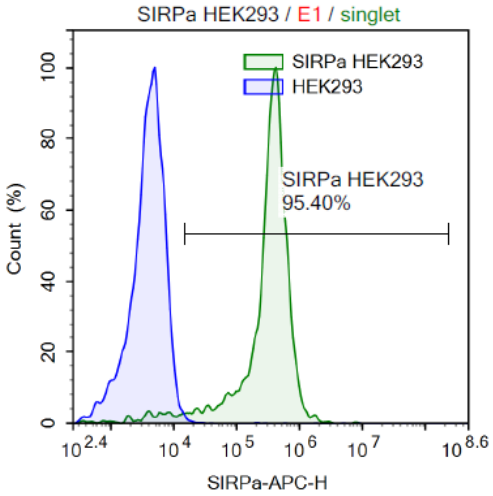

Figure 1. Human SIRPα expression in HEK293 cells
Flow cytometry showed APC-conjugated anti-human CD172a (SIRPα) antibody (Clone REA144; Miltenyi #130-099-785) detects SIRPα on SIRPα / HEK293 cells (green), using HEK293 cells as a negative control (blue).

Vector and Sequence

Human SIRPα (#NM_080792) was cloned into the MCS of pLVX-EF1a-IRES-puro vector (Clontech, #631988).

AA Sequence

MEPAGPAPGRLGPLLCLLLAASCAWSGVAGEEELQVIQPDKSVLVAAGETATLRCTATSLIPVG
PIQWFRGAGPGRELIYNQKEGHFPRVTTVSDLTKRNNMDFSIRIGNITPADAGTYYCVKFRKGS
PDDVEFKSGAGTELSVRAKPSAPVVSGPAARATPQHTVSFTCESHGFSPRDITLKWFKNGNEL
SDFQTNVDPVGESVSYSIHSTAKVVLTREDVHSQVICEVAHVTLQGDPLRGTANLSETIRVPPT
LEVTQQPVRAENQVNVTCQVRKFYPQRLQLTWLENGNVSRTETASTVTENKDGTYNWMSWL
LVNVSAHRDDVKLTCQVEHDGQPAVSKSHDLKVSAHPKEQGSNTAAENTGSNERNIYIVVGVV
CTLLVALLMAALYLVRIRQKKAQGSTSSTRLHEPEKNAREITQDTNDITYADLNLPKGKKPAPQA
AEPNNHTEYASIQTSPQPASEDTLTYADLDMVHLNRTPKQPAPKPEPSFSEYASVQVPRK

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Related Product	Cat. #	Size
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
SIRP-α (CD172a), His-tag (Human)	71145	100 µg
SIRP-α (SIRP alpha), His-tag, Biotin-labeled (Human)	71138	50 µg
SIRP-ɣ (CD172g), Fc fusion, Biotin-labeled (Human)	71236	50 µg
CD47, His-tag (Human)	71127	100 µg
CD47, Fc-Fusion, Strep-Tag (Human) HiP™	71292	100 µg
CD47, Fc fusion (Human) HiP™	71177	100 µg
CD47, Fc fusion, Biotin-labeled (Human) HiP™	71169	50 µg
CD47 - HEK293 Cell Line	71249	2 vials
CD47/TCR-Activator CHO-K1 Cell Line	60602	2 vials
CD47:SIRP-α[Biotinylated] Inhibitor Screening Assay Kit	72044	96 rxns.
CD47:SIRP-γ[Biotinylated] Inhibitor Screening Assay Kit	72059	96 rxns.