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## Data Sheet

### SIRP $\alpha$ / HEK293 Recombinant Cell Line

### Catalog # 60689

#### **Background**

SIRP $\alpha$  (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. SIRP $\alpha$  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 SIRP $\alpha$  (#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<sup>6</sup> cells in 1 ml of 10% DMSO in FBS.

#### **Storage**

Store in liquid nitrogen immediately upon receipt.

#### **Application**

This cell line is useful for SIRP $\alpha$ :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  $\alpha$  (SIRP $\alpha$ ) 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<sub>2</sub>. 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<sub>2</sub>. 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.

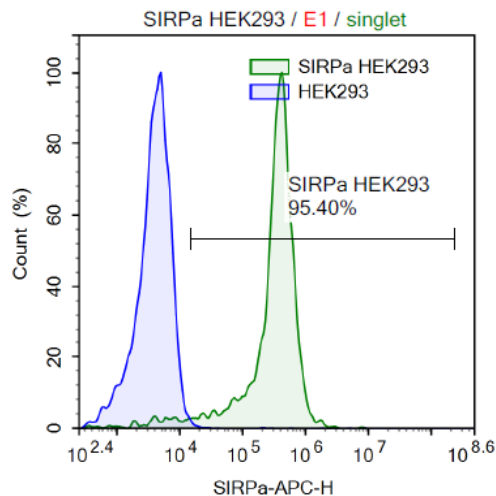
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## Quality Assurance



**Figure 1. Human SIRP $\alpha$  expression in HEK293 cells**

Flow cytometry showed APC-conjugated anti-human CD172a (SIRP $\alpha$ ) antibody (Clone REA144; Miltenyi #130-099-785) detects SIRP $\alpha$  on SIRP $\alpha$  / HEK293 cells (green), using HEK293 cells as a negative control (blue).

## Vector and Sequence

Human SIRP $\alpha$  (#NM\_080792) was cloned into the MCS of pLVX-EF1a-IRES-puro vector (Clontech, #631988).

### AA Sequence

```
MEPAGPAPGRLGPLLCLLLAASCAWSGVAGEEELQVIQPDKSVLVAAGETATLRCTATSLIPVG
PIQWFRGAGPGRELIYNQKEGHFPRVTTVSDLTKRNNMDFSIRIGNITPADAGTYCVKFRKGS
PDDVEFKSGAGTELSVRAKPSAPVVS GPAARATPQHTVSFTCESHGFSPRDITLKWFKNGNEL
SDFQTNVDPVGESVSYSIHSTAKVVLTRDVDHSQVICEVAHVTLQGDPLRGTANLSETIRVPPT
LEVTQQPVRAENQVNVTCQVRKFYPQRLQLTLWLENGNVSR TETASTVTENKDGTYNWMSWL
LVNVAHRDDVKLTCQVEHDGQPAVSKSHDLKVS AHPKEQGSNTAENTGSNERNIYIVGVV
CTLLVALLMAALYLVRIRQKKAQGSTSSTR LHEPEKNAREITQDTNDITYADLNLPKGKKPAPQA
AEPNNHTEYASIQTSPQPA SEDTLTYADLDMVHLNRTPKQPAPKPEPSFSEYASVQVPRK
```

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**Related Product**

<b><u>Related Product</u></b>	<b><u>Cat. #</u></b>	<b><u>Size</u></b>
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-γ (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.

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