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Data Sheet

CD40 - HEK293 Cell Line

Catalog # 71257

Background

CD40, a TNF receptor superfamily member, was initially identified on B lymphocytes. However, other cell types such as monocytes, basophils, dendritic cells, endothelial cells, and epithelial cells have been found to express CD40. High levels of CD40 have also been detected in numerous human cancers, including HCT116, a colon cancer epithelial cell line. Interaction with CD40 ligand (CD40L, CD154) on $CD4^+$ T helper lymphocytes triggers the expression of intercellular adhesion molecule (ICAM) and other pro-inflammatory cytokines. CD40:CD40L signaling simultaneously increases activation of antigen-specific T cells. CD40 also activates NF-κB-dependent signaling in response to lipopolysaccharide (LPS) found on Gram negative bacterial pathogens. Agonistic CD40 monoclonal antibodies have been shown to activate antigen presenting cells (APC), promote anti-tumor T-cell responses, and to foster cytotoxic myeloid cells, suggesting a potential mechanism to control cancer in the absence of T-cell immunity.

Description

Recombinant HEK293 cell line expressing full length human CD40 (Tumor necrosis factor receptor superfamily member 5; TNFRSF5), Genbank Accession No. NP_001241.1. Expression is confirmed by real-time qPCR and Western blot.

Application

Since HEK293 cell line does not express endogenous CD40, the CD40 HEK293 Cell Line is ideal to monitor CD40-specific signaling responses, such as NF-κB activation in response to potential CD40 agonistic monoclonal antibodies.

Host Cell

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

Format

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

Storage

Store in liquid nitrogen immediately upon receipt.

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

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Culture conditions

Frozen Cells: Prepare T-75 culture flask with 20 ml of pre-warmed Thaw Medium 1. 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 the flask with Thaw Medium 1 **(no Hygromycin B)**. Avoid pipetting up and down and gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2. Forty-eight hours after incubation, change to fresh Thaw Medium 1 **(no Hygromycin B)**, without disturbing the attached cells. Continue to change medium every 2-3 days until 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 1F **(contains Hygromycin B)** after the first passage.

Subculture: When cells reached 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 resuspend 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 of 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 2 ml of the cell suspension into a new T75 flask containing pre-warmed 18 ml 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.

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. Diaconu I. *et.al.* (2012) Immune Response is an Important Aspect of the Anti-tumor Effect Produced by a CD40L-Encoding Oncolytic Adenovirus. *Cancer Res.* **72:** 2327.
2. Yacoub D *et.al.* (2013) CD154 is Released from T-cells by a Disintegrin and Metalloproteinase Domain-containing Protein 10 (ADAM10) and ADAM17 in a CD40 Protein-dependent Manner. *J. Biol. Chem.* **288:** 36083.

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Quality Assurance and Functional Analysis

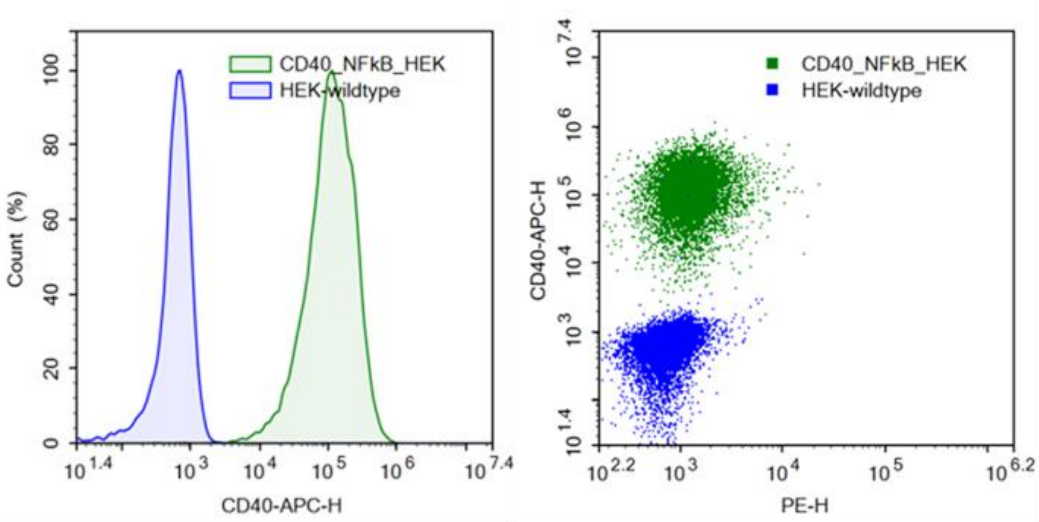

Figure 1. CD40 surface expression in CD40/ HEK293 stable cells. Flow cytometry showed APC-conjugated anti-human CD40 antibody, clone HB14 (Biolegend, Cat #313008) detects CD40-positive cells (green), using naïve HEK293 cells (blue) as negative control.

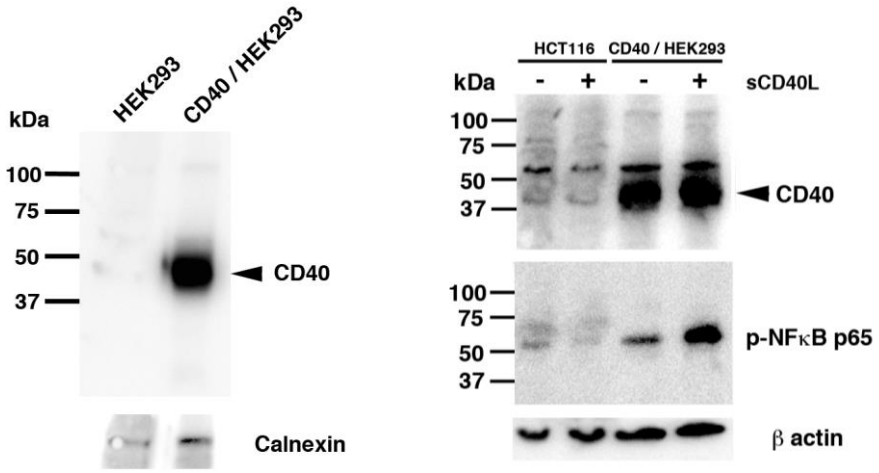

Figure 2. Expression of human CD40 and phospho-NF-κB in response to sCD40L stimulation of HCT116 and CD40/HEK293 cells. (Left) Human CD40 expression in untransfected HEK293 cells and CD40/HEK293 cells, detected by Western blotting, using anti-human CD40 antibody (Santa Cruz Biotechnology, Cat. #sc-975). (**Right**) Cells were seeded in 6 well at semi-confluency overnight and were left untreated (-) or treated (+) with 150 ng/ml of soluble CD40 ligand (CD40L, BPS Cat. #71191) in complete growth media for 18 hours. (**Top blot**) Level of CD40 protein was compared between HCT116 (colonic tumor cells known to

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express CD40), and CD40/HEK293, using anti-CD40 antibody. (**Bottom blot**). Level of CD40 response was measured by NF-κB activation after overnight stimulation with 150 ng/ml of human CD40 ligand (BPS Cat. #71191). NF-κB activation was assessed by p65 Ser536 phosphorylation (NF-κB p65 antibody, Cell Signaling Technology, Cat. #3033). Calnexin and β actin (Cell Signaling Technology, Cat. #2433 and #3700) were used as loading controls.

Vector and sequence
Human CD40 (NP_001241.1; Accession BC012419) was cloned into the MCS of pIREShyg3 vector (Clontech, Cat No. 631620).

MVRLPLQCVLWGCLLTAVHPEPPTACREKQYLINSQCCSLCQPGQKLVSDCTEFTETECLPCG
ESEFLDTWNRETHCHQHKYCDPNLGLRVQQKGTSETDTICTCEEGWHCTSEACESCVLHRSC
SPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPWTSCETKDLVVQQAGTNKTDVVCGP
QDRLRALVVIPIIFGILFAILLVLVFIKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETL
HGCQPVTQEDGKESRISVQERQ

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Related Products

Product	**Cat. #**	**Size**
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
CD40L (CD154), His-tag Protein	71191	100 µg
CD40, Fc fusion Protein	71174	100 µg
CD40/NF-κB Reporter (Luc) - HEK293 Cell Line	60626	2 vials