SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

CD40 NF-κB-Luciferase Reporter (Luc) - HEK293 Stable Cell Line
Catalog # 60626

Description
Recombinant HEK293 cell line expressing full length human CD40 (Tumor necrosis factor receptor superfamily member 5; TNFRSF5). Expression is confirmed by real-time qPCR and western blot. This NF-κB luciferase reporter construct is stably integrated into the genome. The firefly luciferase gene is controlled by 4 copies of NF-kB response element located upstream of the TATA promoter. Following activation by human CD40 ligand, NF-κB transcription factors bind to the DNA response elements to induce transcription of the luciferase gene.

Background
CD40, a TNF receptor superfamily member, was initially identified on B lymphocytes. However, antigen presenting cells (APCs) such as monocytes, basophils, dendritic cells and non-immune cells like endothelial cells and epithelial cells have been found to express CD40. A wide variety of carcinoma cells also over-express CD40. 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. Furthermore, agonistic CD40 monoclonal antibodies have been shown to activate antigen presenting cells (APC) and promote anti-tumor T-cell responses in addition to fostering cytotoxic myeloid cells with the potential to control cancer in the absence of T-cell immunity.

Application
The CD40 NF-κB Reporter HEK293 stable cell line is ideal for high throughput screening (HTS) to identify potential CD40 agonistic monoclonal antibodies and CD40-specific inhibitors.

Host Cell
Human Embryonic Kidney cell line. 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.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Culture Medium

Thaw Medium 1 (BPS Bioscience #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 1A (BPS Bioscience #79528): Thaw Medium 1 (BPS Bioscience Cat. #60187) plus 100 µg/ml Hygromycin B (Thermo Fisher, Cat. #10687010) and 400µg/ml Geneticin®, G418 Sulfate (Thermo Fisher, Cat. # 10131035).

Culture conditions

Frozen Cells: Prepare T-25 culture flask with 10 ml of pre-warmed Thaw Medium 1 **(no hygromycin or G418)**. 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 or G418)**. 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. 48 hours after incubation, change to fresh medium **(no hygromycin or G418)**, 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. Switch to Growth Medium 1A (**with hygromycin or G418)** 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 pre-warmed Growth Medium 1A 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 resuspend cells in 10 ml of pre-warmed Growth Medium 1A. Dispense 5 ml of the cell suspension into a new T75 flask containing pre-warmed 15 ml Growth Medium 1A. 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 Bioscience 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. Li G *et.al.* (2013) Human Genetics in Rheumatoid Arthritis Guides a High-Throughput Drug Screen of the CD40 Signaling Pathway. *PLoS Genet*. **9**: e1003487.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

2. Pontrelli P *et.al.* (2006) CD40L Proinflammatory and Profibrotic Effects on Proximal Tubular Epithelial Cells: Role of NF-kB and Lyn. *JASN*. **17**: 627.
3. Lavorgna A *et.al.* (2014) A Critical Role for IL-17RB Signaling in HTLV-1 Tax-Induced NF-kB Activation and T-Cell Transformation. *PLoS Path*. **10**: e1004418.
4. Moschonas A *et.al*. (2012) CD40 Stimulates a "Feed-Forward" NF-κB- Driven Molecular Pathway That Regulates IFN-β Expression in Carcinoma Cells. *J Immunol*. **188**: 5521.

Quality Assurance

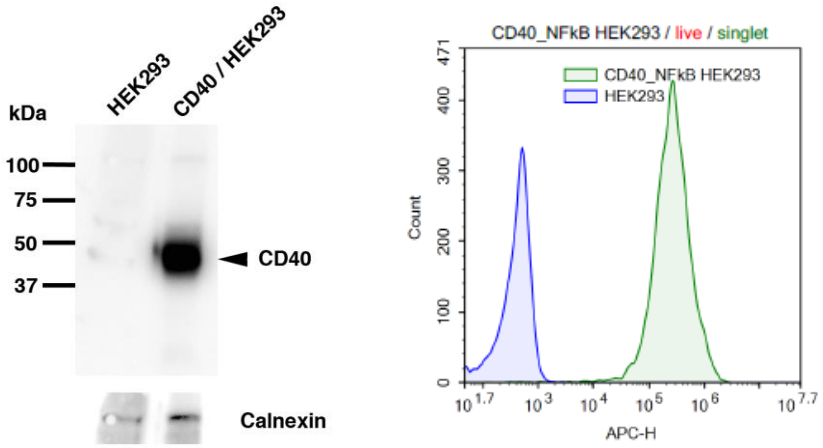


Figure 1. Human CD40 Expression in CD40/HEK293 cells. (Left) Level of CD40 protein was assessed by western blot using a CD40 antibody (Santa Cruz Biotechnology, Cat. #sc-975). Calnexin (Cell Signaling Technology, Cat. #2433) was used as a loading control. (**Right**) Flow cytometry showing CD40 expression using APC anti-human CD40 antibody (Clone 5C3, Biolegend Cat #. 334323)

Functional Analysis

Materials Required but Not Supplied

- Thaw media 1 (BPS Bioscience #60187)
- human TNFα (R&D Systems #210-TA)
- CD40L (BPS Bioscience #71191)
- CD40L antibody (BioLegend #310827)
- 96 well tissue culture treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience #60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Protocol for testing agonists

1. Plate CD40 NF-κB-luciferase reporter HEK293 cells in a 96-well white clear-bottom assay plate at 30,000 cells/well in 50µls/well in Thaw Media 1. Incubate the cells at 37°C, 5% CO2 overnight.

2. The next day prepare TNFα, CD40L, or other desired agonists at 2x in Thaw Media 1.

3. Add 50µls of agonist dilutions to the cells, add 100µls of Thaw Media 1 to cell free control wells (for determining background luminescence) and incubate at 37°C, 5% CO2 for 6 hours.

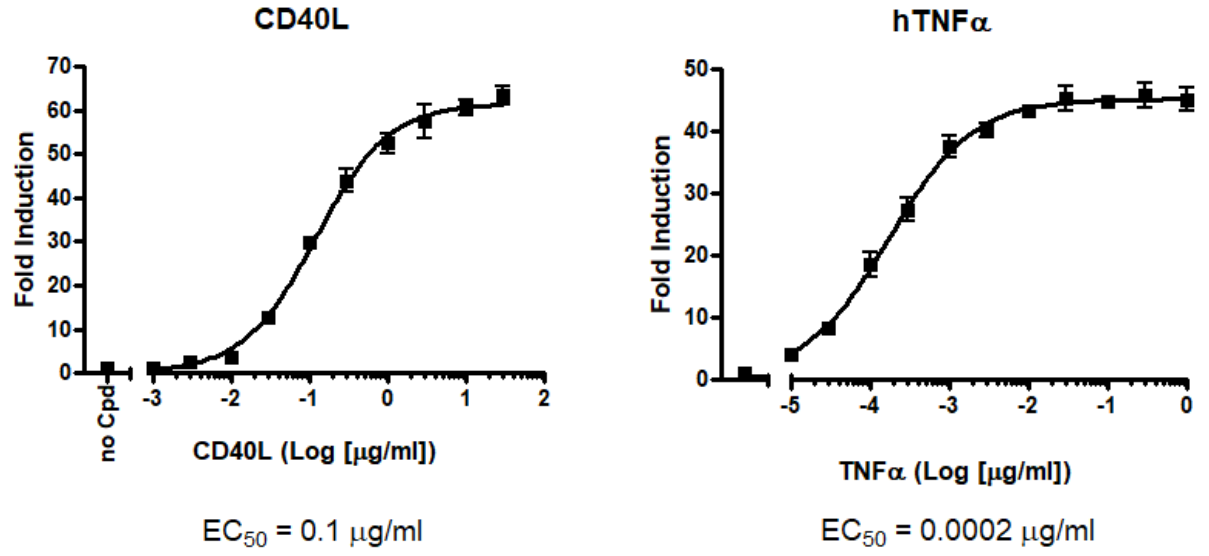
4. After the 6 hour incubation, perform the luciferase assay using the ONE-Step luciferase assay system: Add 100 µl of One-Step luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.

   If using luciferase reagents from other vendors, follow the manufacturer's protocol.

5. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

   The fold induction = background-subtracted luminescence of agonist treated well / average background-subtracted luminescence of untreated control wells.

Figure 2. Response of CD40 NF-κB-luciferase reporter HEK293 to CD40L and hTNFα.



6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Protocol for testing antibodies or inhibitors of CD40L

1. Plate CD40 NF-κB-luciferase reporter HEK293 cells in a 96-well white clear-bottom assay plate at 30,000 cells/well in 50μls/well in Thaw Media 1. Incubate the cells at 37°C, 5% CO2 overnight.

2. The next day prepare the CD40L antibodies or inhibitors at 4x the desired final concentration in Thaw Media 1 and pipet into a 96 well plate.

3. Prepare CD40L at 4x the desired final concentration. In our assays we use a dose equal to the EC50 established for that lot of CD40L.

4. Add a volume of CD40L prepared in step 3 equal to that of the CD40L antibody or inhibitor to the 96 well plate prepared in step 2, mix carefully and incubate at room temperature for 30 minutes. Both the antibody/inhibitor and the CD40L are at 2x in this step.

5. Add 50μls of CD40L:CD40L antibody or inhibitor mix to the cells, add 100μls of Thaw Media 1 to cell free control wells (for determining background luminescence) and incubate at 37°C, 5% CO2 for 6 hours.

6. After the 6 hour incubation, perform the luciferase assay using the ONE-Step luciferase assay system: Add 100 µl of One-Step luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.

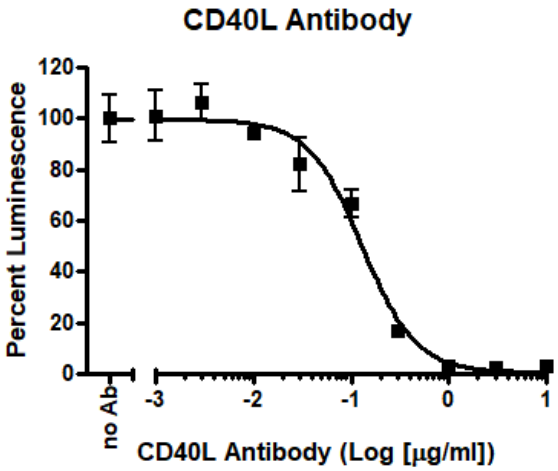
   If using luciferase reagents from other vendors, follow the manufacturer's protocol.

7. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

   The fold induction = background-subtracted luminescence of agonist treated well / average background-subtracted luminescence of untreated control wells.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Figure 3. CD40L antibody inhibition of CD40L response in CD40 NF-κB-luciferase reporter HEK293 cells.



IC_{50} = 0.1 µg/ml

Vector and sequence
NF-κB-Luciferase was cloned into the MCS of pCDNA3.1™ (+) vector (Invitrogen, Cat. #V79020).

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

MVRLPLQCVLWGCLLTAVHPEPPTACREKQYLINSQCCSLCQPGQKLVSDCTEFTETECLPCG
ESEFLDTWNRETHCHQHKYCDPNLGLRVQQKGTSETDTICTCEEGWHCTSEACESCVLHRSC
SPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPWTSCETKDLVVQQAGTNKTDVVCGP
QDRLRALVVIPIIFGILFAILLVLVFIKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETL
HGCQPVTQEDGKESRISVQERQ

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

License Disclosure: Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit the use of this cell line in humans or for therapeutic or drug use. The license does not permit modification of the cell line in any way. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. BPS does not warrant the suitability of the cell line for any particular use and does not accept any liability in connection with the handling or use of the cell line. Modifications of this cell line, transfer to another facility, or commercial use of the cells may require a separate license and additional fees; contact sales@bpsbioscience.com for details. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.

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
CD40L (CD154), His-tag Protein	71191	100 µg
CD40, Fc fusion Protein	71174	100 µg
CD40 HEK293 Stable Cell Line	60625	2 vials
CD40 A549 Stable Cell Line	60626	2 vials