**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-κB 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 10⁶ cells in 1mL 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)

**Complete Growth Medium:** Thaw Medium 1 (BPS 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 and 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 and 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% CO₂. 48 hours after incubation, change to fresh medium (no hygromycin and 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. Begin adding Hygromycin B and G418 to medium (complete growth medium) 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 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 resuspend cells in 10 ml of pre-warmed growth medium. Dispense 5 ml of the cell suspension into a new T75 flask containing pre-warmed 15 ml media. Incubate cells in a humidified 37°C incubator with 5% CO₂. 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**


OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone 1.858.829.3082 Fax 1.858.481.8694
Or you can Email us at: info@bpsbioscience.com
Please visit our website at: www.bpsbioscience.com


---

**Quality Assurance and Functional Analysis**

**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)
Figure 2. CD40/HEK293 NF-κB cells are responsive to human TNFα activation. Cells were seeded in 96 well at 2 x 10⁴ cells per well overnight and were left untreated or treated in complete growth media with human TNFα (Peprotech Cat. #300-01A) for 7 hours. ONE-Step™ Luciferase Assay reagent (BPS Cat. #60690-1) was added and luminescence was recorded using a luminometer. Relative Luminescence Units (RLUs) represent normalized luminescence to untreated cells. Fold increase correlates with RLUs of treated cells with respect to untreated cells. EC₅₀ = 9.954 ng/ml, error bar = SEM, n=3.

Figure 3. CD40/HEK293 NF-κB in response to recombinant human CD40L and anti-CD40L antibody inhibition. (Top) Cells were seeded in 96 well at 2 x 10⁴ cells per well overnight and were left untreated or treated in complete growth media with soluble recombinant human CD40 ligand (CD40L, BPS Cat. #71191) for 7 hours. EC₅₀ = 29.3 µg/ml (Bottom) Cells were seeded overnight and treated with 3 µg/ml of soluble recombinant human CD40L in combination with varying concentrations of anti-CD40L antibody (Biolegend Cat. #310827). After overnight stimulation at 37°C, ONE-Step™ Luciferase Assay reagent (BPS Cat. #60690-1) was added and luminescence was recorded using a luminometer. Relative Luminescence Units (RLUs) represent normalized luminescence to untreated cells. Level of inhibition was evaluated by %
Luciferase Activity (RLUs of treated cells with respect to cells without anti-CD40L antibody treatment). IC$_{50}$ = 39.3 µg/ml. Error bar = SEM, n=3.

**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).

MVRLPLQCVLWGLTAVHPEPPTACREKQYLINSQCSSLCQPGQKLVSQDCTETETECLPCG
ESEFLDTWNRETCHQHKYCDPNLGLRVQQKGTSETDTICTCEEGWHCTSEACEVCNLHRSC
SPGFGVKQIATGVSĐTICEPCPGFFSVSSAFEKCHPWTSCETKDLVQQAGTNKTDVVCGP
QDRRLQVIIIPFIFGIILFALLLVLFVKVKAKKPKTPKHQPQEPQEEINFPPDLPGSNTAAPVQETL
HGCPVQTEGDGKESRISVQERQ

**Related Products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat. #</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thaw Medium 1</td>
<td>60187</td>
<td>100 ml</td>
</tr>
<tr>
<td>ONE-Step™ Luciferase Assay System</td>
<td>60690-1</td>
<td>10 ml</td>
</tr>
<tr>
<td>ONE-Step™ Luciferase Assay System</td>
<td>60690-2</td>
<td>100 ml</td>
</tr>
<tr>
<td>CD40L (CD154), His-tag Protein</td>
<td>71191</td>
<td>100 µg</td>
</tr>
<tr>
<td>CD40, Fc fusion Protein</td>
<td>71174</td>
<td>100 µg</td>
</tr>
<tr>
<td>CD40 HEK293 Stable Cell Line</td>
<td>60625</td>
<td>2 vials</td>
</tr>
<tr>
<td>CD40 A549 Stable Cell Line</td>
<td>60626</td>
<td>2 vials</td>
</tr>
</tbody>
</table>