

Product Sheet

H_CD40(TNFRSF5) Reporter 293 Cell Line

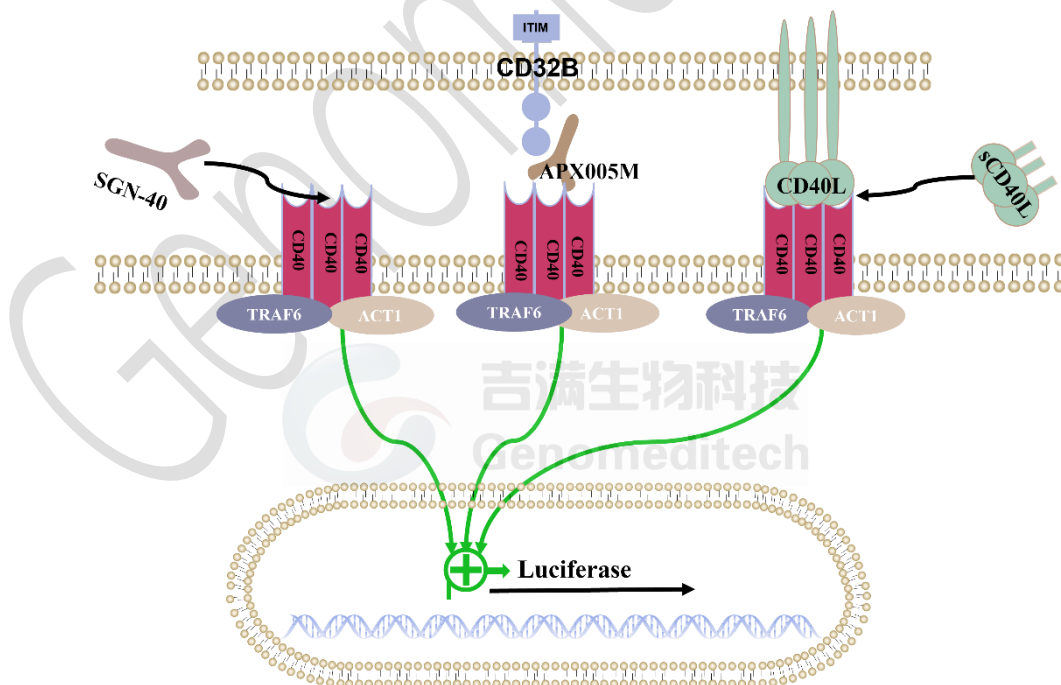
Catalog number: GM-C03829

Version 3.3.1.241216

CD40 is a key cell surface receptor in the TNF receptor superfamily, primarily found on B cells, dendritic cells, macrophages, and some endothelial cells. Its main role is to regulate immune responses by promoting B cell proliferation, differentiation, and antibody production. When it binds to its ligand CD40L (expressed by activated T cells), CD40 activates various signaling pathways that enhance immune function.

CD40 signaling occurs through multiple pathways activated upon CD40L binding, including NF- κ B, MAPK (ERK, JNK, p38 MAPK), and PI3K/Akt. This activation triggers biological responses such as cell proliferation, survival, cytokine secretion, and immune memory formation, making CD40 crucial for regulating immune responses and maintaining immune tolerance.

H_CD40(TNFRSF5) Reporter 293 Cell Line is a clonal stable HEK-293 cell line constructed using lentiviral technology, constitutive expression of the CD40 gene, along with signal-dependent expression of a luciferase reporter gene. When CD40L binds to CD40, it activates downstream signaling pathways, leading to the expression of luciferase. Blockade antibodies can inhibit this signal transmission. The luciferase activity measurement indicates the activation level of the signaling pathway and can thus be used to evaluate the *in vitro* effects of drugs related to CD40.



Specifications

Quantity	5E6 Cells per vial, 1 mL
Product Format	1 vial of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt

Recovery Medium	DMEM+10% FBS+1% P.S
Growth medium	DMEM+10% FBS+1% P.S+4 µg/mL Blasticidin+0.75 µg/mL Puromycin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Adherent
Growth Conditions	37°C, 5% CO ₂

Mycoplasma Testing	The cell line has been screened to confirm the absence of Mycoplasma species.
Safety considerations	Biosafety Level 2
Note	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

Materials

Reagent	Manufacturer/Catalogue No.
DMEM	Gibco/C11995500BT
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/ GM-040404
Puromycin	Genomeditech/ GM-040401
Recombinant Human CD40 Ligand/TNFSF5 Protein	Sino Biological/10239-H08E
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/ GM-040503

Figures

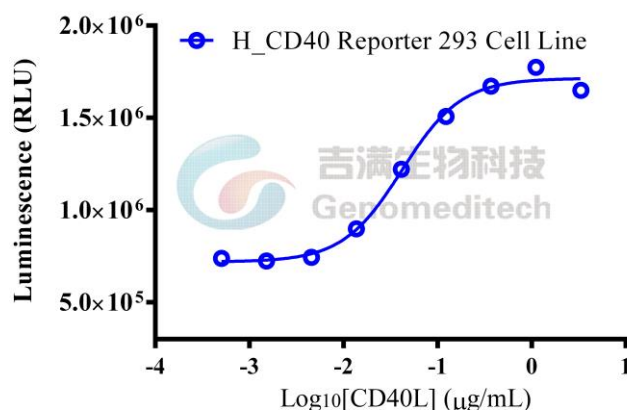


Figure 1 | Response to Recombinant Human CD40 Ligand/TNFSF5 Protein. The H_CD40(TNFRSF5) Reporter 293 Cell Line (Cat. GM-C03829) at a concentration of 1.3E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human CD40 Ligand/TNFSF5 Protein (Sino Biological/10239-H08E) in assay buffer (DMEM + 1% FBS + 1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [2.3]. Data are shown by drug mass concentration.

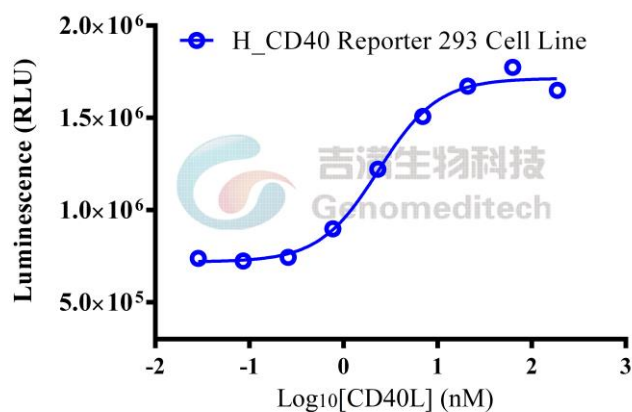


Figure 2 | Response to Recombinant Human CD40 Ligand/TNFSF5 Protein. The H_CD40(TNFRSF5) Reporter 293 Cell Line (Cat. GM-C03829) at a concentration of 1.3E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human CD40 Ligand/TNFSF5 Protein (Sino Biological/10239-H08E) in assay buffer (DMEM + 1% FBS + 1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [2.3]. Data are shown by drug molar concentration.

Cell Recovery

Recovery Medium: DMEM+10% FBS+1% P.S

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C . Storage at -70°C will result in loss of viability.

- a) Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).
- b) Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
- c) Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium and spin at approximately $176 \times g$ for 5 minutes. Discard supernatant.
- d) Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.
- e) Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

- a) Centrifuge at $176 \times g$ for 3 minutes to collect cells.
- b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5×10^6 cells/mL.
- c) Aliquot 1 mL into each vial.
- d) Place the vial in a controlled-rate freezing container and store at -80°C for at least 1 day, then transfer to liquid nitrogen as soon as possible.

Cell passage

Growth medium: DMEM+10% FBS+1% P.S+4 $\mu\text{g}/\text{mL}$ Blasticidin+0.75 $\mu\text{g}/\text{mL}$ Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

- a) Subculturing is necessary when the cell density reaches 80%. It is recommended to perform subculturing at a ratio of 1:3 to 1:4 every 2-3 days. Ensure that the density does not exceed 80%, as overcrowding can lead to reduced viability due to compression.
- b) Remove and discard culture medium.
- c) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
- d) Add 1.0 mL of 0.25% (w/v) Trypsin-EDTA solution to dish and observe cells under an inverted microscope until cell layer is dispersed (usually within 30 to 60 seconds at 37°C).

- e) Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
- f) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
- g) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
- h) Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 - 1:4 is recommended

Medium Renewal: Every 2 to 3 days

Notes

- a) Upon initial thawing, a higher number of dead cells is observed, which is a normal phenomenon. Significant improvement is seen after adaptation. Once the cells reach a stable state, the number of dead cells decreases after subculturing and the cell growth rate becomes stable.
- b) Ensure that the cell density does not exceed 80%, as overcrowding may lead to reduced viability due to compression.

Related Products

CD40: CD40L	
H_CD40(TNFRSF5) Reporter Jurkat Cell Line	Cynomolgus_CD40 CHO-K1 Cell Line
Cynomolgus_CD40L CHO-K1 Cell Line	H_CD40(TNFRSF5) CHO-K1 Cell Line
H_CD40(TNFRSF5) HEK-293 Cell Line	H_CD40L CHO-K1 Cell Line
H_CD40L HEK-293 Cell Line	
Anti-H_CD40 hIgG1 Antibody(APX005M)	Anti-H_CD40 hIgG1 Antibody(ravagalimab)
Anti-H_CD40L hIgG1 Antibody(dapirolizumab)	Anti-H_CD40L hIgG1 Antibody(frexalimab)
Biotinylated Human CD40 Protein; His-Avi Tag	Cynomolgus CD40 Protein; His Tag
Human CD40 Protein; His Tag	Human CD40L Protein; His Tag
IFN- α	
IFNα Reporter HEK-293 Cell Line	IFNα Reporter MDCK Cell Line
IFNα Reporter THP1 Cell Line	
BCMA:BAFFR:TACI	
H_BAFFR Jurkat Blockade Reporter Cell Line	H_BAFFR Reporter Cell Line
H_BCMA Reporter Cell Line	H_TACI Reporter Cell Line
Cynomolgus_BCMA CHO-K1 Cell Line	H_BCMA CHO-K1 Cell Line
H_BCMA HEK-293 Cell Line	
Anti-BAFF hIgG1 Antibody(belimumab)	Anti-BAFFR hIgG1 Antibody(ianalumab)
Anti-BCMA hIgG1 Antibody(Belantamab)	Anti-BCMA hIgG1 Antibody(SEA-BCMA)
Anti-BCMA hIgG4 Antibody(BCMB69)	
Biotinylated Human BAFF Protein; His-Avi Tag	Cynomolgus BAFF Protein; His Tag
Human BAFF Protein; His Tag	Mouse BAFF Protein; His Tag
BDCA2(CLEC4C)	

H_BDCA2 Reporter Jurkat Cell Line	Cynomolgus_BDCA2 CHO-K1 Cell Line
Cynomolgus_BDCA2 Jurkat Cell Line	H_BDCA2 CHO-K1 Cell Line
H_BDCA2 HEK-293 Cell Line	H_BDCA2 Jurkat Cell Line
Anti-H_BDCA2 hIgG1 Antibody(Litifilimab)	
Cynomolgus BDCA2 Protein; His Tag	Human BDCA2 Protein; His Tag

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