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# **Product Sheet**

## H\_CD40L HEK-293 Cell Line

Catalog number: GM-C35531

Version 3.3.1.241128

Description	H_CD40L HEK-293 Cell Line is a clonal stable HEK-293 cell line that constitutively expresses the human CD40L gene, constructed using lentiviral technology.	
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	
Target	Human_CD40L	
Gene ID/Uniprot ID	P29965	
Host Cell	HEK-293	
<b>Recovery Medium</b>	DMEM+10% FBS+1% P.S	
Growth medium	DMEM+10% FBS+1% P.S+0.75 µg/mL Puromycin	
Note	None	
Freezing Medium	90% FBS+10% DMSO	
Growth properties	Adherent	
Growth Conditions	37°C, 5% CO <sub>2</sub>	
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.	



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## **Materials**

Reagent	Manufacturer/Catalogue No.
DMEM	Gibco/C11995500BT
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Puromycin	Genomeditech/GM-040401
Anti-H_CD40L hIgG1 Antibody(dapirolizumab)	Genomeditech/GM-83977AB

## Figures

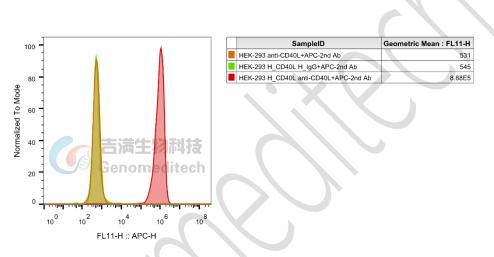
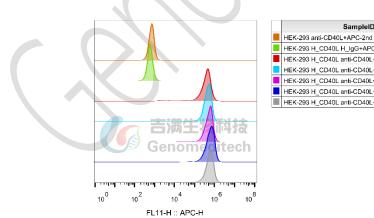


Figure 1 | H\_CD40L HEK-293 Cell Line (Cat. GM-C35531) was determined by flow cytometry using Anti-H\_CD40L hIgG1 Antibody(dapirolizum) (Cat. GM-83977AB).



SampleID	Geometric Mean : FL11-H
HEK-293 anti-CD40L+APC-2nd Ab	715
HEK-293 H_CD40L H_IgG+APC-2nd Ab	606
HEK-293 H_CD40L anti-CD40L+APC-2nd Ab CP5	302242
HEK-293 H_CD40L anti-CD40L+APC-2nd Ab CP10	4.26E5
HEK-293 H_CD40L anti-CD40L+APC-2nd Ab CP15	4.76E5
HEK-293 H_CD40L anti-CD40L+APC-2nd Ab CP20	5.62E5
HEK-293 H_CD40L anti-CD40L+APC-2nd Ab CP25	5.46E5

Figure 2 | The passage stability of the H\_CD40L HEK-293 Cell Line (Cat. GM-C35531) was determined by flow cytometry using Anti-H\_CD40L hIgG1 Antibody(dapirolizum) (Cat. GM-83977AB).

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## **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  $-70^{\circ}$ C. Storage at  $-70^{\circ}$ 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 x 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<sub>2</sub> 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 x g for 3 minutes to collect cells.
- b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 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+0.75 µg/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.
- 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.

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- 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.

## Sequence

CD40L(CD40LG) P29965

MIETYNQTSPRSAATGLPISMKIFMYLLTVFLITQMIGSALFAVYLHRRLDKIEDERNLHEDFVFMKTIQRCNT GERSLSLLNCEEIKSQFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYT MSNNLVTLENGKQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQ QSIHLGGVFELQPGASVFVNVTDPSQVSHGTGFTSFGLLKL

## **Related Products**

CD40: CD40L				
H_CD40(TNFRSF5) Reporter Jurkat Cell Line				
Cynomolgus_CD40L CHO-K1 Cell Line				
H_CD40(TNFRSF5) HEK-293 Cell Line				
Anti-H_CD40 hIgG1 Antibody(ravagalimab)				
Anti-H_CD40L hIgG1 Antibody(frexalimab)				
Cynomolgus CD40 Protein; His Tag				
Human CD40L Protein; His Tag				
IFN-α				
IFNa Reporter MDCK Cell Line				
BCMA:BAFFR:TACI				
H_BCMA Reporter Cell Line				
Cynomolgus_BCMA CHO-K1 Cell Line				
H_BCMA HEK-293 Cell Line				
Anti-BAFFR hIgG1 Antibody(ianalumab)				
Anti-BCMA hIgG1 Antibody(SEA-BCMA)				

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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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