Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_Nkp46 Reporter Jurkat Cell Line

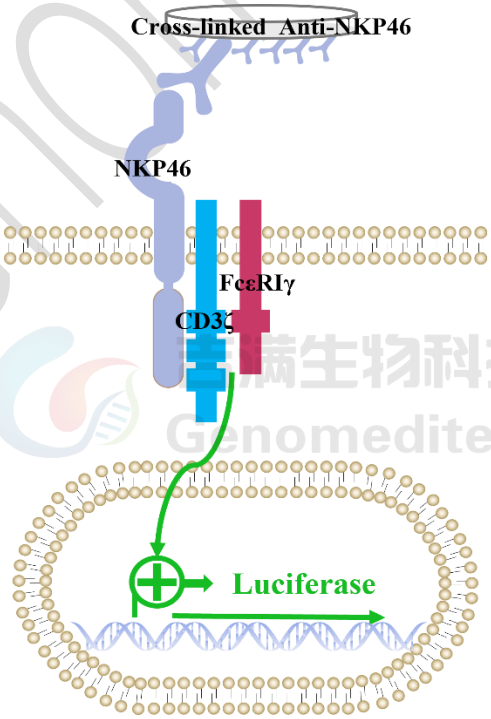
Catalog number: GM-C17954

Version 3.1.1.240905

NKp46, encoded by the NCR1 gene, is also known as lymphocyte antigen 94 homolog, NK cell activation receptor, natural killer cell p46-related protein, and CD335. It is a 46 kDa glycoprotein belonging to the immunoglobulin (Ig) superfamily and is frequently expressed on tumor-infiltrating lymphocytes. Antibodies that activate NKp46 can induce not only the cytotoxic effects of NK cells but also the release of cytokines.

NKp46 interacts through its positively charged transmembrane domain with signaling adaptor proteins CD3ζ and Fc epsilon R1 gamma, which carry ITAMs (immunoreceptor tyrosine-based activation motifs). These signaling proteins can form disulfide-linked homodimers and heterodimers, participating in the signal transduction process and enhancing the function of NK cells.

H_Nkp46 Reporter Jurkat Cell Line is a clonal stable Jurkat cell line constitutively expressing the Nkp46 receptor, an adapter membrane molecule and a luciferase reporter gene. The addition of coated NKP46 antibody agonists stimulates Nkp46 to bind with CD3ζ and Fc epsilon R1 gamma, activating downstream reporter genes and inducing luciferase expression. This system can be used to evaluate the in vitro effects of antibodies targeting NKG2D.



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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	RPMI 1640+10% FBS+1% P.S
Growth medium	RPMI 1640+10% FBS+1% P.S+0.75 μg/mL Puromycin+400 μg/mL G418+3.5 μg/mL Blasticidin
Note	None
Freezing Medium	90% FBS+10%DMSO
Growth properties	Suspension
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.

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Figures

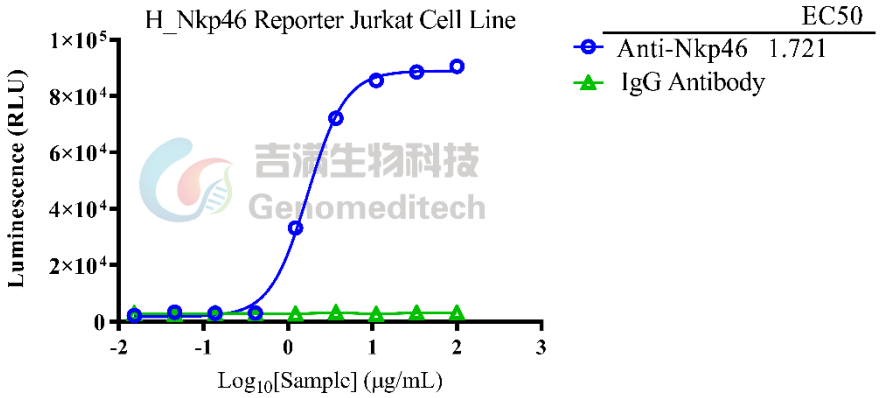


Figure 1 | Response to Anti-NCR1(NKP46) hIgG1 Antibody. H_Nkp46 Reporter Jurkat Cell Line (Genomeditech/GM-C17954) at a concentration of 1E5 cells/well in a 96-well format. The wells were coated overnight with serial dilutions of Anti-NCR1(NKP46) hIgG1 Antibody (A26-BhlgG1) (Genomeditech/GM-29003AB) and Human IgG1 Isotype Control (Anti-RSV) (Genomeditech/GM-47471AB) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). After coating, the cells were added and incubated for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Genomeditech/GM-040503C). The maximum induction fold was approximately [40.2]. Data are shown by drug mass concentration.

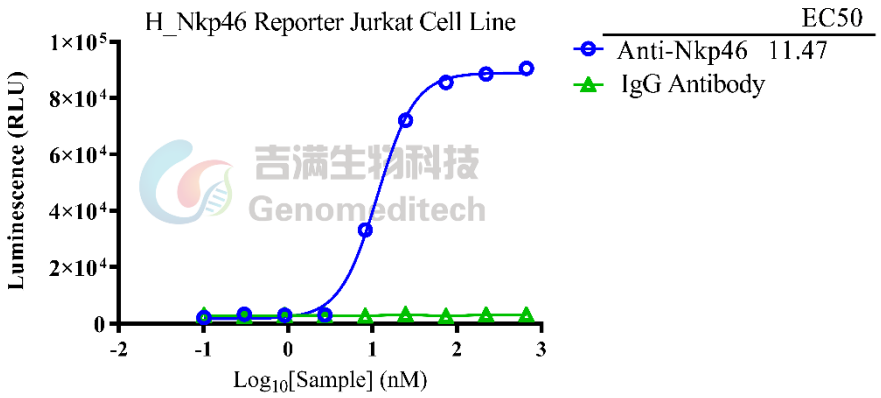
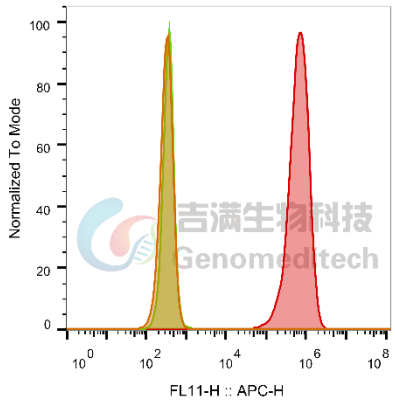


Figure 2 | Response to Anti-NCR1(NKP46) hIgG1 Antibody. H_Nkp46 Reporter Jurkat Cell Line (Genomeditech/GM-C17954) at a concentration of 1E5 cells/well in a 96-well format. The wells were coated overnight with serial dilutions of Anti-NCR1(NKP46) hIgG1 Antibody (A26-BhlgG1) (Genomeditech/GM-29003AB) and Human IgG1 Isotype Control (Anti-RSV) (Genomeditech/GM-47471AB) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). After coating, the cells were added and incubated for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Genomeditech/GM-040503C). The maximum induction fold was approximately [40.2]. Data are shown by drug molar concentration.



SampleID	Geometric Mean : FL11-H
Jurkat anti-NKP46+APC-2nd Ab	326
H_NKP46 Reporter Jurkat H_IgG+APC-2nd Ab	370
H_NKP46 Reporter Jurkat anti-NKP46+APC-2nd Ab	6.09E5

Figure 3 | H_Nkp46 Reporter Jurkat Cell Line was determined by flow cytometry using Anti-NCR1(NKP46) hIgG1 Antibody(A26-BhlgG1) (Genomeditech/GM-29003AB).

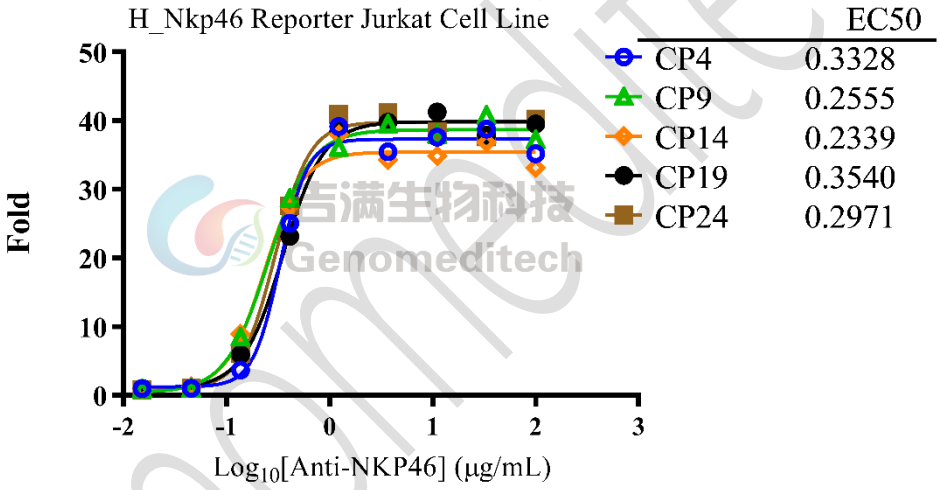


Figure 4 | Response to Anti-NCR1(NKP46) hIgG1 Antibody. The passage 4, 9, 14,19 and 24 of H_Nkp46 Reporter Jurkat Cell Line (Genomeditech/GM-C17954) at a concentration of 1E5 cells/well in a 96-well format. The wells were coated overnight with serial dilutions of Anti-NCR1(NKP46) hIgG1 Antibody (A26-BhlgG1) (Genomeditech/GM-29003AB) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). After coating, the cells were added and incubated for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Genomeditech/GM-040503C). Data are shown by drug mass concentration.

Cell Recovery

Recovery Medium: RPMI 1640+10% FBS+1% P.S

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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 x g for 5 minutes. Discard supernatant.
d) Resuspend cell pellet with the recommended complete medium. And dispense the suspension into 1 - 2 T-25 culture flasks.
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 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: RPMI 1640+10% FBS+1% P.S+0.75 μg/mL Puromycin+400 μg/mL G418+3.5 μg/mL Blasticidin

Approximately 48-72 hours after the initial thawing, the cells can be passaged for the first time. After this initial passage, the culture medium can be adjusted to growth medium supplemented with antibiotics. If cells are not passaged within 48 hours, it is recommended to add some fresh recovery medium and place the flask horizontally.

a) When the cell density reaches 1.5 - 2E6 cells/mL, subculture the cells. Do not allow the cell density to exceed 2E6 cells/mL.
b) It is recommended to use T-25 flasks for subculturing.
c) These cells are suspension cells, and it is recommended to use the "half-medium change" method to maintain optimal cell conditions during passaging.
d) During passaging, you can directly add fresh growth medium to the culture flask, gently pipette to resuspend the cells, and then transfer the cell suspension to a new T-25 flask for continued culture.

Subcultivation Ratio: Maintain cultures at a cell concentraion between 3E5 and 1E6 viable cells/mL.

Medium Renewal: Every 2 to 3 days

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Notes

a) These cells are sensitive to density, so please ensure that the cell density is maintained within an appropriate range during culture and subculturing.

b) During the first passage, pay attention to the nutrient supply; if not subculturing, make sure to add fresh recovery medium every other day as needed.

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