Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_NKG2D Blockade Reporter Jurkat Cell Line

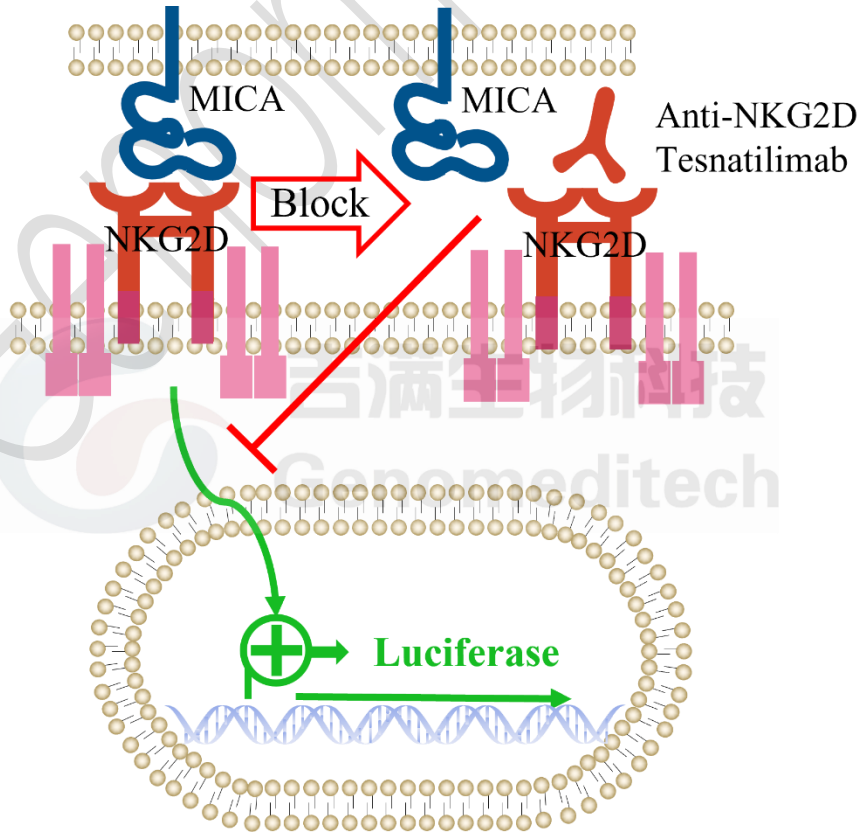
Catalog number: GM-C27755

Version 3.3.1.241129

NKG2D is an activating receptor (transmembrane protein) that belongs to the C-type lectin-like receptor NKG2 family. It is encoded by the KLRK1 (killer cell lectin-like receptor K1) gene, which is located in the NK gene complex (NKC) on chromosome 6 in mice and chromosome 12 in humans.

In NK cells, two main receptors control the balance between cellular activation and inhibition: one recognizes MHC I (HLA-A, B, C), and the other recognizes non-MHC I molecules. Receptors that recognize MHC I are NKG2A and NKG2C, while NKG2D and NCR primarily recognize non-MHC I molecules. NKG2D is a type II membrane protein, expressed as a dimer on the cell membrane. The ligands for NKG2D primarily include MICA/MICB and ULBP1-6. When ligands bind to NKG2D, the YXXM motif on the DAP10 protein can recruit GRB2 and PI3K, thereby activating PLCγ and subsequently activating NK cells.

H_NKG2D Blockade Reporter Jurkat Cell Line is a clonal stable Jurkat cell line constructed using lentiviral technology, constitutively expressing NKG2D chimeric receptor and some adapter membrane molecules, along with signal-dependent expression of a luciferase reporter gene. When MICA binds to NKG2D, it activates downstream signaling pathways, leading to the expression of luciferase. Blockade antibodies can inhibit this signal transmission. The luciferase readout represents the activation level of the signaling pathway and can thus be used for evaluating the in vitro effects of neutralizing antibody of NKG2D.



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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	RPMI 1640+10% FBS+1% P.S
Growth medium	RPMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin+400 μg/mL G418+200 μg/mL Hygromycin+0.75 μg/mL Puromycin
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.

Materials

Reagent	Manufacturer/Catalogue No.
RPMI 1640	gibco/C11875500BT
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Puromycin	Genomeditech/GM-040401
Blasticidin	Genomeditech/GM-040404
G418	Genomeditech/GM-040402
Hygromycin	Genomeditech/GM-040403
H_MICA CHO-K1 Cell Line	Genomeditech/GM-C22346
Anti-H_KLRK1(NKG2D) hIgG4 Antibody(Tesnatilimab)	Genomeditech/GM-28955AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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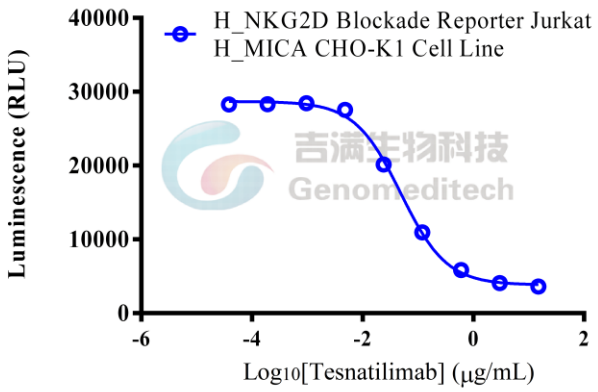
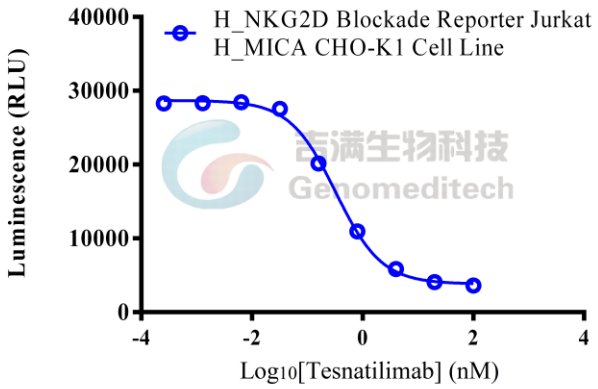
Figures



	H_NKG2D Blockade Reporter Jurkat H_MICA CHO-K1 Cell Line
IC50	0.04889

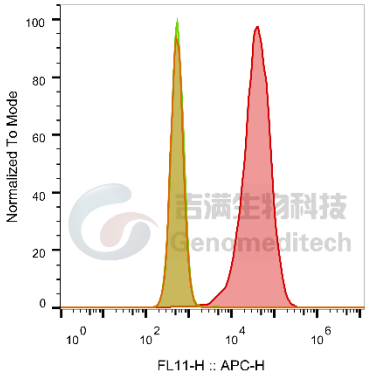
Figure 1 | Response to Anti-H_KLRK1(NKG2D) hIgG4 Antibody(Tesnatilimab). Serial dilutions of Anti-H_KLRK1(NKG2D) hIgG4 Antibody(Tesnatilimab) (Cat. GM-28955AB) was incubated with 1E5 cells/well of the H_NKG2D Blockade Reporter Jurkat Cell Line (Cat. GM-C27755) in a 96-well plate for 1 hour in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_MICA CHO-K1 Cell Line (Cat. GM-C22346) at a density of 1E4 cells/well in a 96-well format, and incubate for 6 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 [7.8]. Data are shown by drug mass concentration.



	H_NKG2D Blockade Reporter Jurkat H_MICA CHO-K1 Cell Line
IC50	0.3259

Figure 2 | Response to Anti-H_KLRK1(NKG2D) hIgG4 Antibody(Tesnatilimab). Serial dilutions of Anti-H_KLRK1(NKG2D) hIgG4 Antibody(Tesnatilimab) (Cat. GM-28955AB) was incubated with 1E5 cells/well of the H_NKG2D Blockade Reporter Jurkat Cell Line (Cat. GM-C27755) in a 96-well plate for 1 hour in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_MICA CHO-K1 Cell Line (Cat. GM-C22346) at a density of 1E4 cells/well in a 96-well format, and incubate for 6 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 [7.8]. Data are shown by drug molar concentration.



SampleID	Geometric Mean : FL11-H
Jurkat anti-NKG2D+APC-2nd Ab	540
H_NKG2D Blockade Reporter Jurkat H_IgG+APC-2nd Ab	557
H_NKG2D Blockade Reporter Jurkat anti-NKG2D+APC-2nd Ab	36252

Figure 3 | H_NKG2D Blockade Reporter Jurkat Cell Line (Cat. GM-C27755) was determined by flow cytometry using Anti-H_KLRK1(NKG2D) hIgG4 Antibody(Tesnatilimab) (Cat. GM-28955AB).

Cell Recovery

Recovery Medium: RPMI 1640+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 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+3.5 μg/mL Blasticidin+400 μg/mL G418+200 μg/mL Hygromycin+0.75 μg/mL Puromycin

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

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.

Related Products

NKG2A:HLA-E	
H_HLA-E HEK-293 Cell Line	H_NKG2A H_CD94 CHO-K1 Cell Line
Anti-H_KLRC1 hIgG4 Antibody(Monalizumab)	Anti-HLA-E hIgG1 Antibody(ABX-0020)
MICA;MICB	
Cynomolgus_MICA(AAO24115) CHO-K1 Cell Line	Cynomolgus_MICA(Q2MGE0-1) CHO-K1 Cell Line
Cynomolgus_MICB CHO-K1 Cell Line	H_MICA CHO-K1 Cell Line
H_MICA HEK-293 Cell Line	H_MICA*001 Luciferase B16-F10 Cell Line
H_MICA*001 MC38 Cell Line	H_MICA*008 CHO-K1 Cell Line
H_MICB CHO-K1 Cell Line	H_MICB HEK-293 Cell Line
Anti-MICA/MICB hIgG1 Antibody(36 NF G236A)	Anti-MICA/MICB mIgG2a Antibody(7C6)
Anti-MICA/MICB mIgG2a Antibody(PDI-01)	

NKG2D	
Cynomolgus_NKG2D CHO-K1 Cell Line	H_NKG2D CHO-K1 Cell Line
H_NKG2D HEK-293 Cell Line	
Anti-H_KLRK1(NKG2D) hIgG4 Antibody(Tesnatilimab)	Anti-NKG2D hIgG1 Antibody(A49MI)

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