Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_LILRB2(ILT4) Reporter Jurkat Cell Line

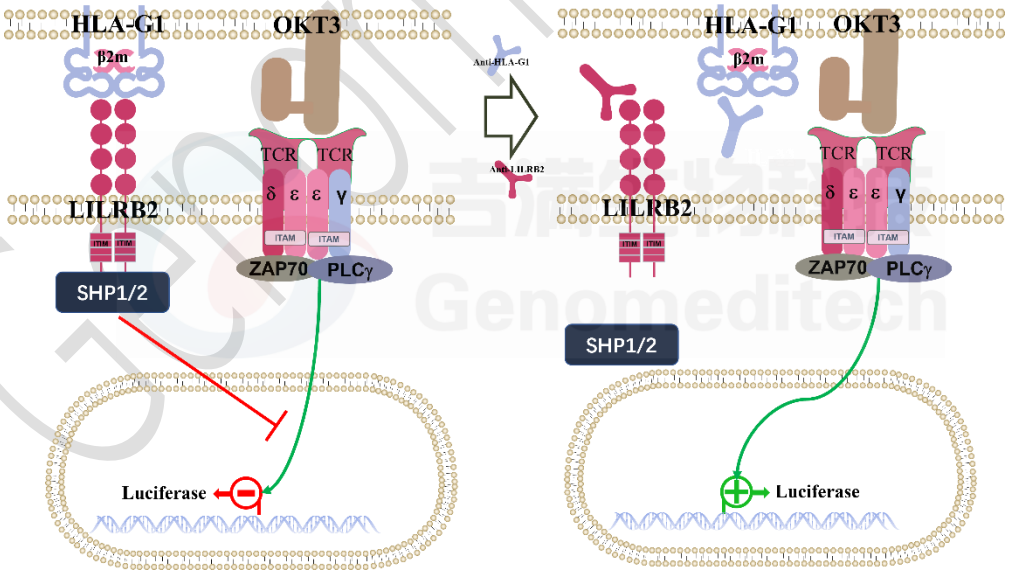
Catalog number: GM-C16968

Version 3.3.1.241206

LILRB2, a member of the leukocyte Ig-like receptor subfamily B (LILRB), is a single-pass transmembrane glycoprotein with extracellular Ig-like domains and intracellular ITIM motifs. Upon binding to ligands, LILRB2 activates ITIM motifs, triggering signals that inhibit T cell activation.

HLA-G, a non-classical MHC class I molecule, is crucial for fetal-maternal tolerance and acts as an immune checkpoint. By interacting with LILRB1 (ILT2) and LILRB2 (ILT4), HLA-G suppresses cytotoxic T cells, NK cells, and B cells, induces T cell anergy, and promotes regulatory T cell (Treg) development. HLA-G on antigen-presenting cells (APCs), such as MDSCs or tolerogenic dendritic cells, further promotes T cell hyporesponsiveness and Treg differentiation.

H_LILRB2(ILT4) Reporter Jurkat Cell Line is a clonal stable Jurkat cell line constructed using lentiviral technology, constitutive expression of the LILRB2(ILT4) gene, along with signal-dependent expression of a luciferase reporter gene. The reporter cell line is co-cultured with the H_HLA-G1 OKT3 CHO-K1 Cell Line. The binding of HLA-G1 to LILRB2(ILT4) inhibit T cell signaling. By adding Anti-LILRB2 and Anti-HLA-G1 antibodies, the interaction of HLA-G1 to LILRB2 is blocked, thereby restoring T cell signaling. The luciferase readout indicates the activation level of the signaling pathway, allowing evaluation of the in vitro effects of LILRB1 related drugs.



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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+0.75 μg/mL Puromycin+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.

Materials

Reagent	Manufacturer/Catalogue No.
RPMI 1640	VivaCell/C3010-0500
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Puromycin	Genomeditech/GM-040401
Blasticidin	Genomeditech/GM-040404
H_HLA-G1 OKT3 CHO-K1 Cell Line	Genomeditech/GM-C16834
Anti-H_LILRB2(ILT4) hIgG4 Antibody(MK-4830)	Genomeditech/GM-27362AB
Anti-H_HLA-G1 hIgG1 Antibody(38373)	Genomeditech/GM-28208AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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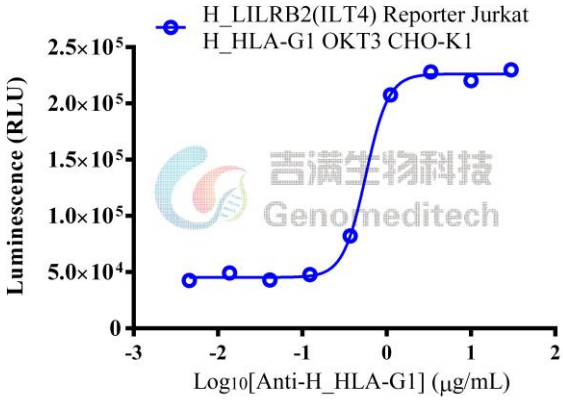
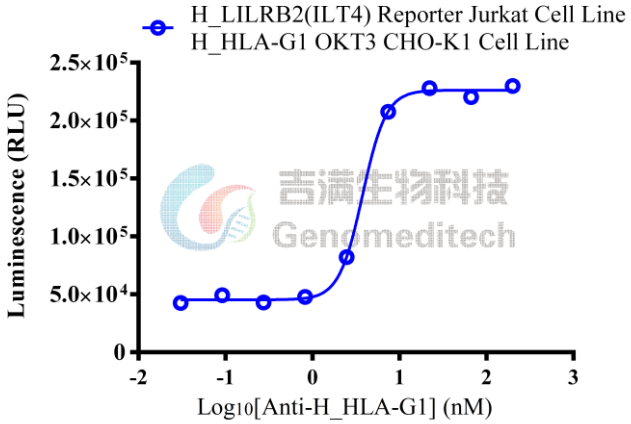
Figures



	H_LILRB2(ILT4) Reporter Jurkat H_HLA-G1 OKT3 CHO-K1
EC50	0.5652

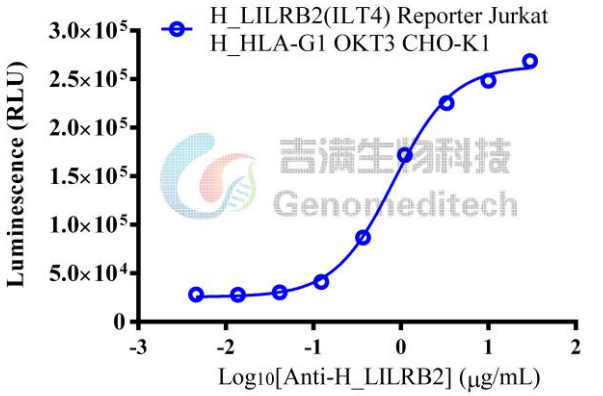
Figure 1 | Response to Anti-H_HLA-G1 hIgG1 Antibody(38373). H_HLA-G1 OKT3 CHO-K1 Cell Line (Cat. GM-C16834) was seeded at a density of 2E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-H_HLA-G1 hIgG1 Antibody(38373) (Cat. GM-28208AB) was incubated with H_HLA-G1 OKT3 CHO-K1 Cell Line for 1 hour. Subsequently, the H_LILRB2(ILT4) Reporter Jurkat Cell Line (Cat. GM-C16968) at a concentration of 1E5 cells/well was added, and the coculture proceeded for an additional 5 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated maximum blocking folds of approximately [4.5]. Data are shown by drug mass concentration.



	H_LILRB2(ILT4) Reporter Jurkat
EC50	3.768

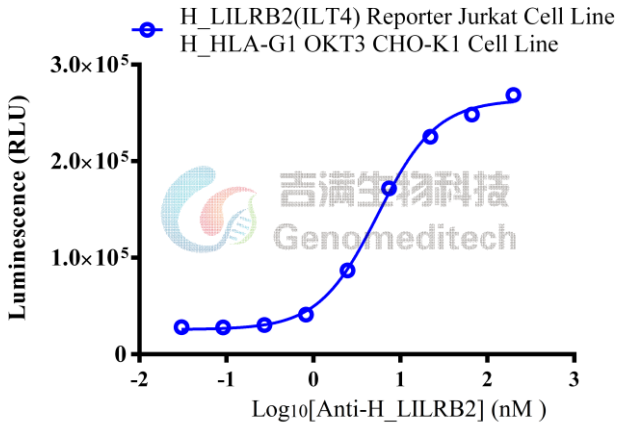
Figure 2 | Response to Anti-H_HLA-G1 hIgG1 Antibody(38373). H_HLA-G1 OKT3 CHO-K1 Cell Line (Cat. GM-C16834) was seeded at a density of 2E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-H_HLA-G1 hIgG1 Antibody(38373) (Cat. GM-28208AB) was incubated with H_HLA-G1 OKT3 CHO-K1 Cell Line for 1 hour. Subsequently, the H_LILRB2(ILT4) Reporter Jurkat Cell Line (Cat. GM-C16968) at a concentration of 1E5 cells/well was added, and the coculture proceeded for an additional 5 hours. Firefly luciferase

activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated maximum blocking folds of approximately [4.5]. Data are shown by drug molar concentration.



	H_LILRB2(ILT4) Reporter Jurkat H_HLA-G1 OKT3 CHO-K1
EC50	0.8266

Figure 3 | Response to Anti-H_LILRB2(ILT4) hIgG4 Antibody(MK-4830). H_HLA-G1 OKT3 CHO-K1 Cell Line (Cat. GM-C16834) was seeded at a density of 2E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-H_LILRB2(ILT4) hIgG4 Antibody(MK-4830) (Cat. GM-27362AB) were incubated with 1E5 cells/well of the H_LILRB2(ILT4) Reporter Jurkat Cell Line (Cat. GM-C16968) in a 96-well plate for 1 hour, and then added to the pre-seeded cells. The mixture was incubated for an additional 5 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated maximum blocking folds of approximately [11.2]. Data are shown by drug mass concentration.



	H_LILRB2(ILT4) Reporter Jurkat
EC50	5.510

Figure 4 | Response to Anti-H_LILRB2(ILT4) hIgG4 Antibody(MK-4830). H_HLA-G1 OKT3 CHO-K1 Cell Line (Cat. GM-C16834) was seeded at a density of 2E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-H_LILRB2(ILT4) hIgG4 Antibody(MK-4830) (Cat. GM-27362AB) were incubated with

1E5 cells/well of the H_LILRB2(ILT4) Reporter Jurkat Cell Line (Cat. GM-C16968) in a 96-well plate for 1 hour, and then added to the pre-seeded cells. The mixture was incubated for an additional 5 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated maximum blocking folds of approximately [11.2]. Data are shown by drug molar concentration.

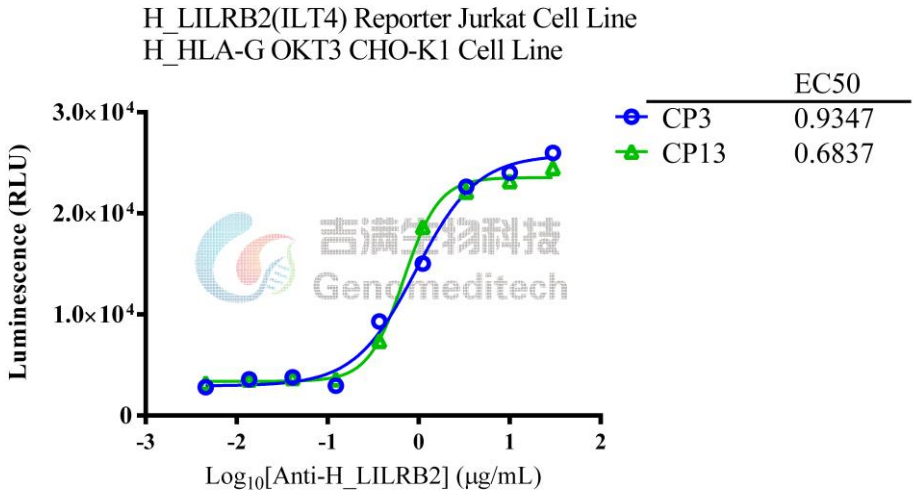
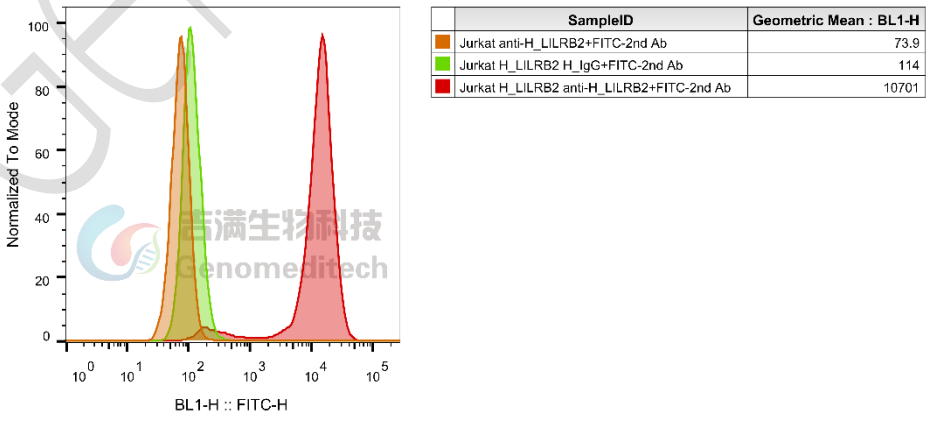


Figure 5 | Response to Anti-H_LILRB2(ILT4) hIgG4 Antibody(MK-4830). H_HLA-G1 OKT3 CHO-K1 Cell Line (Cat. GM-C16834) was seeded at a density of 2E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-H_LILRB2(ILT4) hIgG4 Antibody(MK-4830) (Cat. GM-27362AB) were incubated with 1E5 cells/well of the H_LILRB2(ILT4) Reporter Jurkat Cell Line (Cat. GM-C16968) CP3 and CP13 in a 96-well plate for 1 hour, and then added to the pre-seeded cells. The mixture was incubated for an additional 5 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug mass concentration.



SampleID	Geometric Mean : BL1-H
Jurkat anti-H_LILRB2+FITC-2nd Ab	73.9
Jurkat H_LILRB2 H_IgG+FITC-2nd Ab	114
Jurkat H_LILRB2 anti-H_LILRB2+FITC-2nd Ab	10701

Figure 6 | H_LILRB2(ILT4) Reporter Jurkat Cell Line (Cat. GM-C16968) was determined by flow cytometry using Anti-H_LILRB2(ILT4) hIgG4 Antibody(1E1) (Cat. GM-27362AB).

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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+0.75 μg/mL Puromycin+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

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

LILRB2(ILT4)	
H_LILRB2(ILT4) CHO-K1 Cell Line	H_LILRB2(ILT4) HEK-293 Cell Line
Anti-H_LILRB2(ILT4) hIgG4 Antibody(MK-4830)	
LILRB1(ILT2)	
H_LILRB1(ILT2) Reporter Jurkat Cell Line	H_LILRB1(ILT2) CHO-K1 Cell Line
H_LILRB1(ILT2) HEK-293 Cell Line	Rhesus_LILRB1 CHO-K1 Cell Line
Anti-LILRB1(ILT2) mIgG1 Antibody(12D12)	
LILRB4(ILT3)	
H_LILRB4(ILT3) CHO-K1 Cell Line	H_LILRB4(ILT3) HEK-293 Cell Line
LILRB5	
H_LILRB5 CHO-K1 Cell Line	
LILRB3(ILT5)	
H_LILRB3 Reporter Jurkat Cell Line	H_LILRB3(ILT5) CHO-K1 Cell Line
H_LILRB3(ILT5) HEK-293 Cell Line	
Anti-LILRB3 hIgG1 Antibody(7C5)	

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