

# **Product Sheet**

# H\_LILRB1(ILT2) Reporter Jurkat Cell Line

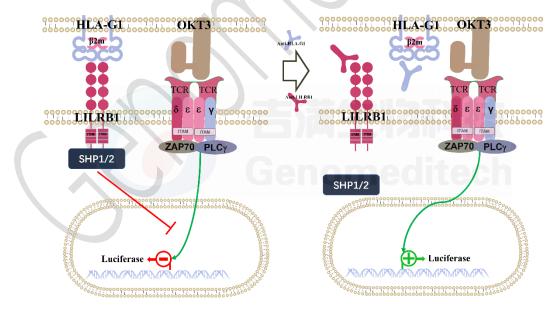
Catalog number: GM-C15867

Version 3.3.1.241203

HLA-G1 (Human Leukocyte Antigen G1) is a class I molecule of the major histocompatibility complex (MHC), primarily expressed in the placenta and certain tumors. It regulates immune cell functions by binding to receptors like KIR2DL4, LILRB1, and LILRB2. LILRB1 (Leukocyte Immunoglobulin-Like Receptor B1) is a key receptor in the human immune system.

When HLA-G1 binds to LILRB1, LILRB1 mediates inhibitory signals through its intracellular ITIM domain. The ITIM domain is phosphorylated, recruiting inhibitory signaling molecules such as SHP-1 and SHP-2, which in turn inhibit downstream activation signaling pathways.

H\_LILRB1(ILT2) Reporter Jurkat Cell Line is a clonal stable Jurkat cell line constructed using lentiviral technology, constitutive expression of the LILRB1 gene and some adapter membrane molecules, 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 LILRB1 inhibit T cell signaling. By adding Anti-LILRB1 and Anti-HLA-G1 antibodies, the interaction of HLA-G1 to LILRB1 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.





## 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+0.75 µg/mL Puromycin		
Note	None		
Freezing Medium	90% FBS+10% DMSO		
Growth properties	Suspension		
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.		
Materials			

#### **Materials**

Reagent	Manufacturer/Catalogue No.
RPMI 1640	gibco/C11875500BT
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
H_HLA-G1 OKT3 CHO-K1 Cell Line	Genomeditech/GM-C16834
Anti-LILRB1(ILT2) mIgG1 Antibody(12D12)	Genomeditech/GM-27366AB
Anti-H_HLA-G1 mIgG2a Antibody	Genomeditech/GM-38392AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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#### Figures

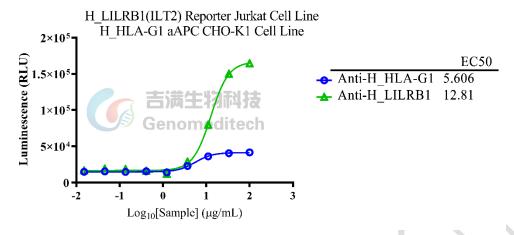


Figure 1 | Response to Anti-H\_HLA-G1 mIgG2a Antibody and Anti-H\_LILRB1(ILT2) mIgG1 Antibody. Serial dilutions of Anti-H\_HLA-G1 mIgG2a Antibody (Cat. GM-38392AB) were incubated with 2E4 cells/well of H\_HLA-G OKT3 CHO-K1 Cell Line (Cat. GM-C16834) for 1 hour, then add to 1E5 cells/well of H\_LILRB1(ILT2) Reporter Jurkat Cell Line (Cat. GM-C15867) incubate for 5 hours. Serial dilutions of Anti-H\_LILRB1(ILT2) mIgG1 Antibody (Cat. GM-27366AB) were incubated with 1E5 cells/well of H\_LILRB1(ILT2) mIgG1 Antibody (Cat. GM-27366AB) were incubated with 1E5 cells/well of H\_LILRB1(ILT2) Reporter Jurkat Cell Line (Cat. GM-C15867) for 1 hour, then were added to 2E4 cells/well of H\_HLA-G OKT3 CHO-K1 Cell Line (Cat. GM-C16834) incubate for 5 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit. Data are shown by drug mass concentration.

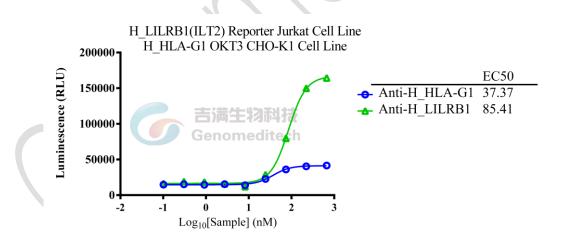


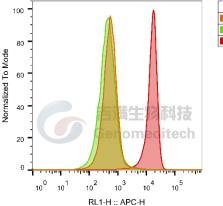
Figure 2 | Response to Anti-H\_HLA-G1 mIgG2a Antibody and Anti-H\_LILRB1(ILT2) mIgG1 Antibody. Serial dilutions of Anti-H\_HLA-G1 mIgG2a Antibody (Cat. GM-38392AB) were incubated with 2E4 cells/well of H\_HLA-G OKT3 CHO-K1 Cell Line (Cat. GM-C16834) for 1 hour, then add to 1E5 cells/well of H\_LILRB1(ILT2) Reporter Jurkat Cell Line (Cat. GM-C15867) incubate for 5 hours. Serial dilutions of Anti-H\_LILRB1(ILT2) mIgG1 Antibody (Cat. GM-27366AB) were incubated with 1E5 cells/well of H\_LILRB1(ILT2) Reporter Jurkat Cell Line (Cat. GM-C15867) for 1 hour, then were added to 2E4 cells/well of H\_HLA-G OKT3 CHO-K1 Cell Line (Cat. GM-C16834) incubate for 5 hours. Firefly

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luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit. Data are shown by drug molar concentration.



SampleID	Geometric Mean : RL1-H
Jurkat anti-LILRB1+APC-2nd Ab	524
Jurkat H_LILRB1 M_IgG+APC-2nd Ab	417
Jurkat H_LILRB1 anti-LILRB1+APC-2nd Ab	14719

Figure 3 | H\_LILRB1(ILT2) Reporter Jurkat Cell Line (Cat. GM-C15867) was determined by flow cytometry using Anti-H\_LILRB1(ILT2) mIgG1 Antibody (Cat. GM-27366AB).

#### **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  $-70^{\circ}$ 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 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<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.

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- 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+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**

LILRB2(ILT4)				
H_LILRB2(ILT4) Reporter Jurkat Cell Line	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) CHO-K1 Cell Line	H_LILRB1(ILT2) HEK-293 Cell Line			
Rhesus_LILRB1 CHO-K1 Cell Line				
Anti-LILRB1(ILT2) mIgG1 Antibody(12D12)	Anti-LILRB1/LILRB2 hIgG1 Antibody			
LILRB4(ILT3)				
H_LILRB4(ILT3) CHO-K1 Cell Line	H_LILRB4(ILT3) HEK-293 Cell Line			
LILRB5				

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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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