Genomeditech (Shanghai) Co.,Ltd.
Order: +86 021-68455258/50432826/50432825
Toll-free: +86 400 627 9288
Email: service@genomeditech.com

Product Sheet

H_BTLA PD-1 Reporter Cell Line

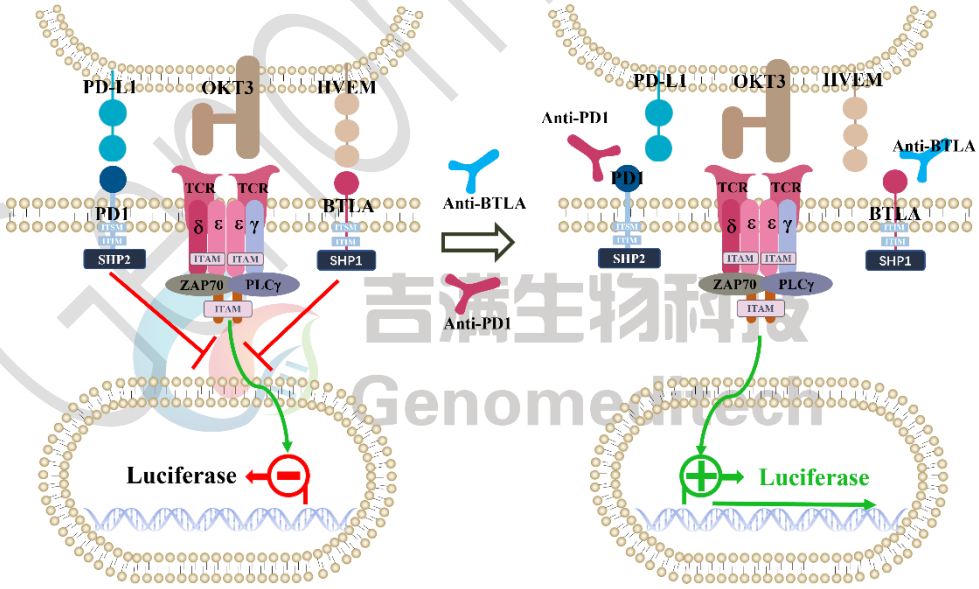
Catalog number: GM-C31560

Version 3.3.1.241122

BTLA (B and T lymphocyte attenuator) and HVEM (herpesvirus entry mediator) are key immune regulatory proteins that form an important regulatory pathway. BTLA, as an inhibitory co-stimulatory receptor, suppresses T cell activation and immune responses when bound to HVEM.

PD-1 (programmed cell death protein 1) is a receptor on activated T cells, B cells, and NK cells that inhibits T cell activation by binding to its ligands PD-L1 and PD-L2. PD-L1, primarily found on tumor and immune cells, promotes immune evasion. The PD-1/PD-L1 pathway is crucial for regulating immune tolerance and inhibiting autoimmune responses, maintaining immune balance.

The H_BTLA PD-1 Reporter Cell Line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of the human BTLA and PD-1, and exhibits signal-dependent expression of a luciferase reporter gene. It is developed on a base cell line where HVEM and H_LIGHT have been knocked out. The reporter cell line is co-cultured with the H_HVEM PD-L1 aAPC CHO-K1 cell line. The binding of BTLA to HVEM and PD-1 to PD-L1 both inhibit T cell signaling. By adding Anti-BTLA and Anti-PD1 antibodies, the interactions of BTLA-HVEM and PD-1-PD-L1 are 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 BTLA and PD-1 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+3.5 μg/mL Blasticidin+400 μg/mL Bleomycin+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	VivaCell/C3010-0500
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Bleomycin	Genomeditech/GM-040407
Puromycin	Genomeditech/GM-040401
H_HVEM aAPC CHO-K1 Cell Line	Genomeditech/GM-C25499
H_HVEM PD-L1 aAPC CHO-K1 Cell Line	Genomeditech/GM-C31561
aAPC(OKT3) PDL1 CHO-K1 Cell Line	Genomeditech/GM-C05269
Anti-BTLA hIgG4 Antibody(22B3)	Genomeditech/GM-50103AB
Anti-PD1 hIgG4 Antibody(Pembrolizumab)	Genomeditech/GM-52674AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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Figures

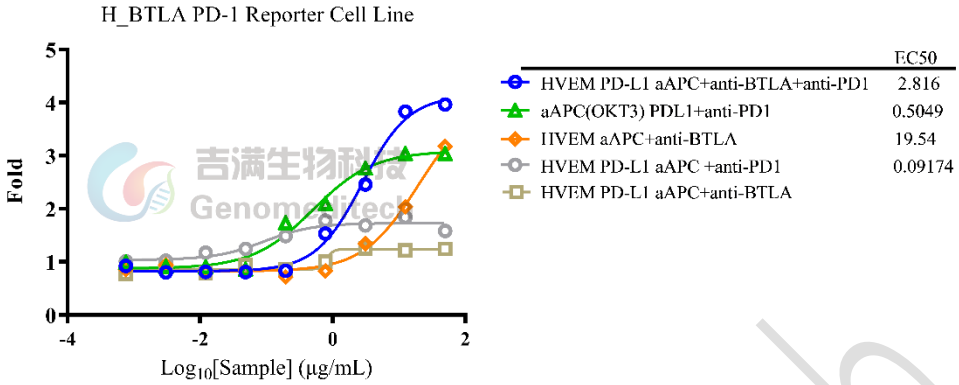


Figure 1 | Response to Anti-PD1 hIgG4 Antibody (Pembrolizumab) and Anti-BTLA hIgG4 Antibody(22B3). Serial dilutions of both antibodies were incubated with 1E5 cells/well of the H_BTLA PD-1 Reporter Cell Line in a 96-well plate for 1 hour. Subsequently added to the H_HVEM PD-L1 aAPC CHO-K1 Cell Line plated overnight at a concentration of 1E4 cells/well, followed by an additional 6 hours. The control group included co-culturing the H_BTLA PD-1 Reporter Cell Line with the aAPC (OKT3) PDL1 CHO-K1, H_HVEM aAPC CHO-K1, and H_HVEM PD-L1 aAPC CHO-K1 Cell Lines, followed by the corresponding antibodies. Firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit, resulting in a maximum blocking fold of approximately [3.9].

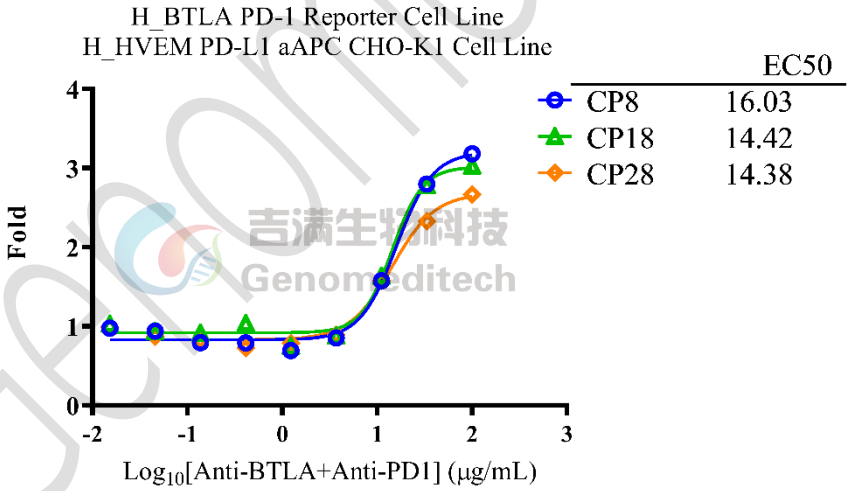
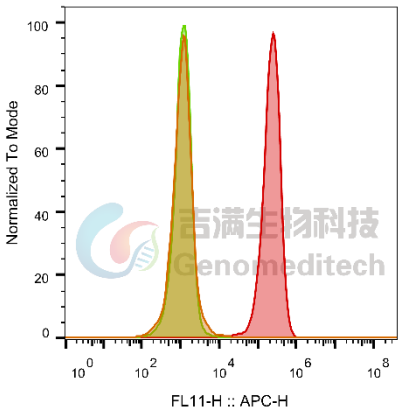
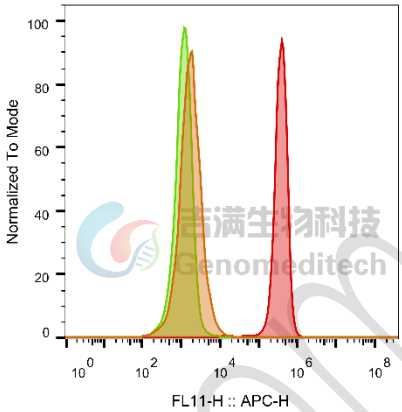


Figure 2 | The passage stability of response to Anti-PD1 hIgG4 Antibody(Pembrolizumab) and Anti-BTLA hIgG4 Antibody(22B3). The passage 8, 18 and 28 passage of 1E5 cells/well H_BTLA PD-1 Reporter Cell Line (Cat. GM-C31560) with serial dilutions of the Anti-PD1 hIgG4 Antibody(Pembrolizumab) (Cat. GM-52674AB), Anti-BTLA hIgG4 Antibody(22B3) (Cat. GM-50103AB) for 1 hour. Subsequently added to the H_HVEM PD-L1 aAPC CHO-K1 Cell Line (Cat. GM-C31561) plated overnight at a concentration of 1E4 cells/well, followed by an additional 6 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug mass concentration.



SampleID	Geometric Mean : FL11-H
Null anti-BTLA+APC-2nd Ab	1101
H_BTLA PD-1 Reporter H_IgG+APC-2nd Ab	1120
H_BTLA PD-1 Reporter anti-BTLA+APC-2nd Ab	210188

Figure 3 | H_BTLA PD-1 Reporter Cell Line (Cat. GM-C31560) was determined by flow cytometry using Anti-BTLA hIgG4 Antibody(22B3) (Cat. GM-50103AB).



SampleID	Geometric Mean : FL11-H
Null anti-PD-1+APC-2nd Ab	1694
H_BTLA PD-1 Reporter H_IgG+APC-2nd Ab	1125
H_BTLA PD-1 Reporter anti-PD-1+APC-2nd Ab	348772

Figure 4 | H_BTLA PD-1 Reporter Cell Line (Cat. GM-C31560) was determined by flow cytometry using Anti-PD1 hIgG4 Antibody(Pembrolizumab) (Cat. GM-52674AB).

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.

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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 Bleomycin+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.

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Related Products

BTLA:HVEM:LIGHT	
H_BTLA Reporter Cell Line	H_HVEM aAPC CHO-K1 Cell Line
H_HVEM PD-L1 aAPC CHO-K1 Cell Line	H_HVEM Reporter Jurkat Cell Line
Cynomolgus_BTLA HEK-293 Cell Line	H_BTLA CHO-K1 Cell Line
H_BTLA HEK-293 Cell Line	H_LIGHT(TNFSF14) CHO-K1 Cell Line
H_TNFRSF14(HVEM) CHO-K1 Cell Line	
Anti-BTLA hIgG4 Antibody(22B3)	Anti-BTLA hIgG4 Antibody(Icatolimab)
Anti-TNFRSF14(HVEM) hIgG4 Antibody	Anti-TNFSF14 hIgG4 Antibody

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