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

Product Sheet

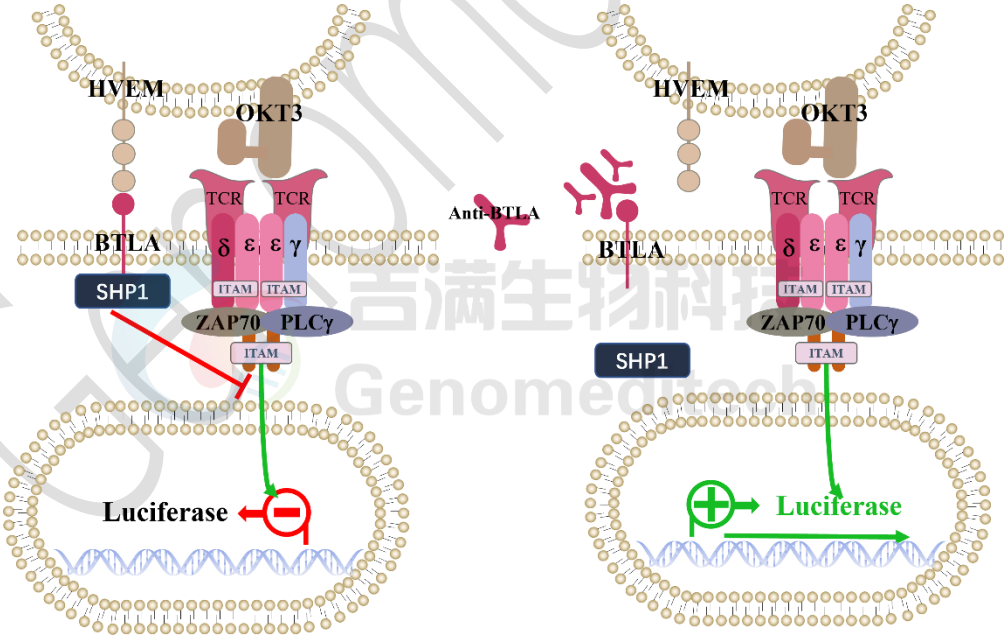
H_BTLA Reporter Cell Line

Catalog number: GM-C28122

Version 3.3.1.241205

B and T lymphocyte attenuator (BTLA; CD272) is an Ig superfamily member that negatively regulates immune cell activation, primarily expressed on B cells, T cells, and dendritic cells. Its natural ligand, herpetic virus entry mediator (HVEM; TNFRSF14), is part of the tumor necrosis factor receptor superfamily. BTLA and HVEM modulate T cell and antigen-presenting cell functions through their cell surface expression. BTLA binding to HVEM inhibits T cell proliferation, downregulates the activation marker CD25, and suppresses cytokine production (IFN-γ, IL-2, IL-4, IL-10) without causing apoptosis, leading to reduced T cell activation and proliferation.

H_BTLA Reporter Cell Line is a clonal stable cell line that constitutively expresses BTLA, along with signal-dependent expression of a luciferase reporter gene, developed on a base cell line where HVEM and H_LIGHT has been knocked out. When H_BTLA Reporter Cell Line are co-cultured with H_HVEM aAPC CHO-K1 Cell Line, HVEM binds to BTLA, recruits SHP1, and inhibits TCR signaling, thereby suppressing the expression of luciferase. By adding anti-BTLA antibodies to block the BTLA-HVEM interaction, TCR signaling is restored, allowing for normal expression of luciferase.The luciferase readout represents the activation level of the signaling pathway and can thus be used to evaluate the in vitro effects of drugs related to BTLA.



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

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.

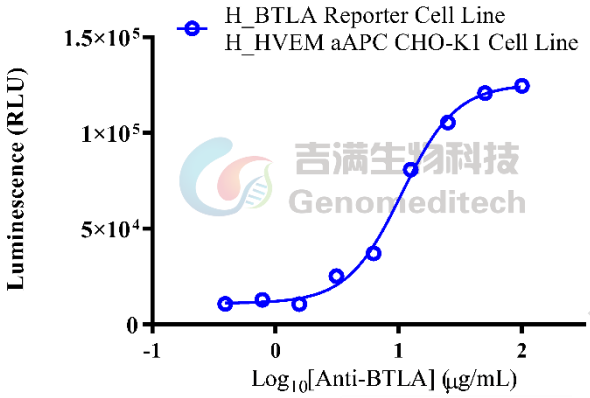
Materials

Reagent	Manufacturer/Catalogue No.
RPMI 1640	VivaCell/C3010-0500
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
G418	Genomeditech/GM-040402
Hygromycin	Genomeditech/GM-040403
Puromycin	Genomeditech/GM-040401
Clear Flat-Bottom Immuno Nonsterile 96-Well Plates	Thermo/442404
H_HVEM aAPC CHO-K1 Cell Line	Genomeditech/GM-C25499
Anti-BTLA hIgG4 Antibody(22B3)	Genomeditech/GM-50103AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

Genomeditech

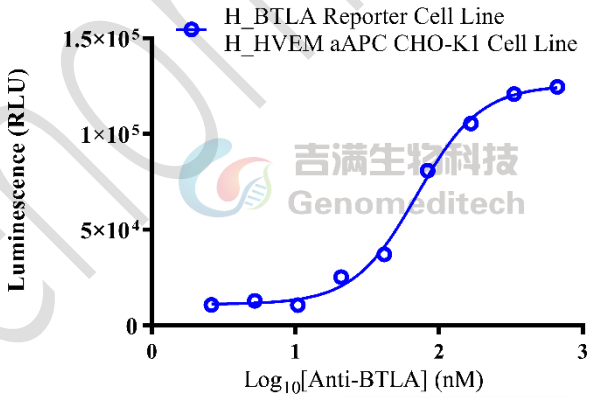
Genomeditech (Shanghai) Co.,Ltd.
Order: +86 021-68455258/50432826/50432825
Toll-free: +86 400 627 9288
Email: service@genomeditech.com

Figures



	H_BTLA Reporter Cell Line H_HVEM aAPC CHO-K1 Cell Line
EC50	10.55

Figure 1 | Response to Anti-BTLA hIgG4 Antibody(22B3). Serial dilutions of the Anti-BTLA hIgG4 Antibody(22B3) (Cat. GM-50103AB) was incubated with 1E5 cells/well of the H_BTLA Reporter Cell Line (Cat. GM-C28122) in a 96-well plate for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S), then were added to 2E4 cells/well of H_HVEM aAPC CHO-K1 Cell Line (Cat. GM-C25499) for 7 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [10.2]. Data are shown by drug mass concentration.



	H_BTLA Reporter Cell Line H_HVEM aAPC CHO-K1 Cell Line
EC50	70.35

Figure 2 | Response to Anti-BTLA hIgG4 Antibody(22B3). Serial dilutions of the Anti-BTLA hIgG4 Antibody(22B3) (Cat. GM-50103AB) was incubated with 1E5 cells/well of the H_BTLA Reporter Cell Line (Cat. GM-C28122) in a 96-well plate for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S), then were added to 2E4 cells/well of H_HVEM aAPC CHO-K1 Cell Line (Cat. GM-C25499) for 7 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [10.2]. Data are shown by drug molar concentration.

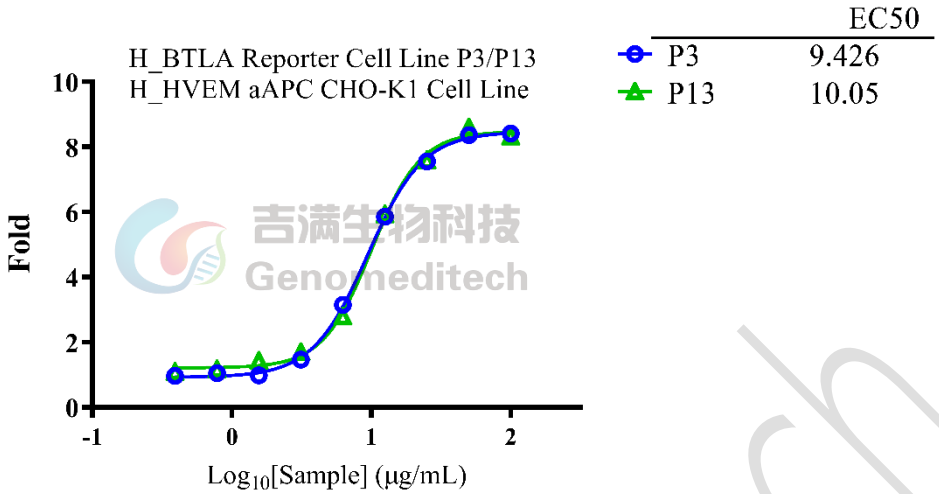


Figure 3 | The passage stability of response to Anti-BTLA hIgG4 Antibody(22B3). Incubate the CP3 and CP13 passage of 1E5 cells/well H_BTLA Reporter Cell Line (Cat. GM-C28122) with serial dilutions of the Anti-BTLA hIgG4 Antibody (22B3) (Cat. GM-50103AB) for 1 hour, then were added to 2E4 cells/well of H_HVEM aAPC CHO-K1 Cell Line (Cat. GM-C25499) and incubate in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 7 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.

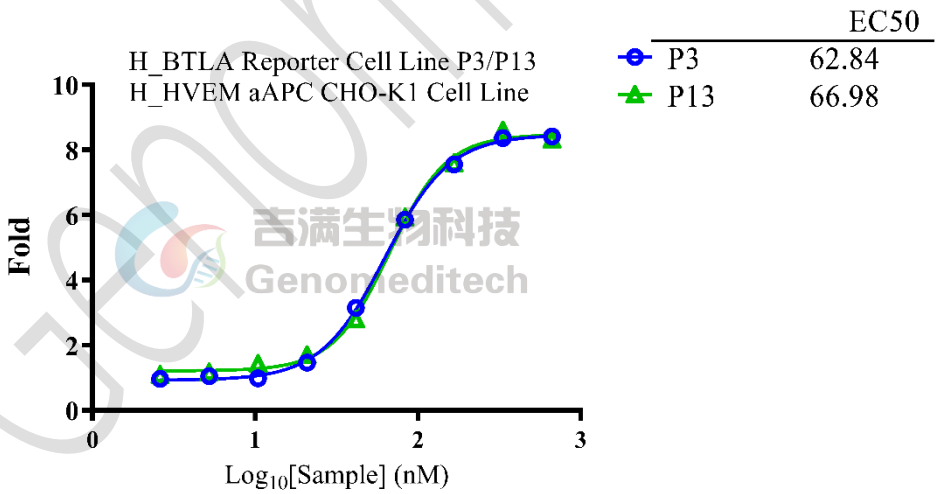
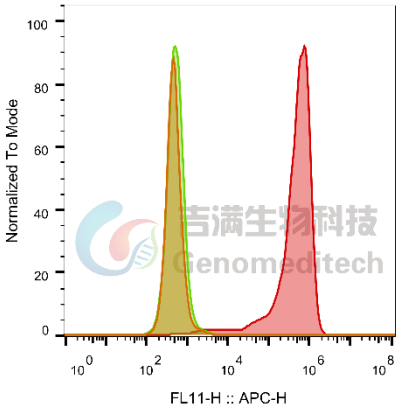


Figure 4 | The passage stability of response to Anti-BTLA hIgG4 Antibody(22B3). Incubate the CP3 and CP13 passage of 1E5 cells/well H_BTLA Reporter Cell Line (Cat. GM-C28122) with serial dilutions of the Anti-BTLA hIgG4 Antibody (22B3) (Cat. GM-50103AB) for 1 hour, then were added add 2E4 cells/well of H_HVEM aAPC CHO-K1 Cell Line (Cat. GM-C25499) and incubate in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug molar concentration.



SampleID	Geometric Mean : FL11-H
Cell anti-BTLA+APC-2nd Ab	491
H_BTLA Reproter Cell H_IgG+APC-2nd Ab	543
H_BTLA Reproter Cell anti-BTLA+APC-2nd Ab	384446

Figure 5 | H_BTLA Reporter Cell Line (Cat. GM-C28122) was determined by flow cytometry using Aanti-BTLA hIgG4 Antibody(22B3) (Cat. GM-50103AB).

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

BTLA:HVEM:LIGHT	
H_BTLA PD-1 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

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

Limited Use License Agreement

Genomeditech (Shanghai) Co., Ltd grants to the Licensee all intellectual property rights, exclusive, non-transferable, and non-sublicensable rights of the Licensed Materials; Genomeditech (Shanghai) Co., Ltd will retain ownership of the Licensed Materials, cell line history packages, progeny, and the Licensed Materials including modified materials.

Between Genomeditech (Shanghai) Co., Ltd, and Licensee, Licensee is not permitted to modify cell lines in any way. The Licensee shall not share, distribute, sell, sublicense, or otherwise provide the Licensed Materials, or progenitors to third parties such as laboratories, departments, research institutions, hospitals, universities, or biotechnology companies for use other than for the purpose of outsourcing the Licensee's research.

Please refer to the Genomeditech Cell Line License Agreement for details.

Genomeditech