Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_CTLA4 PD-1 Reporter Cell Line

Catalog number: GM-C26486

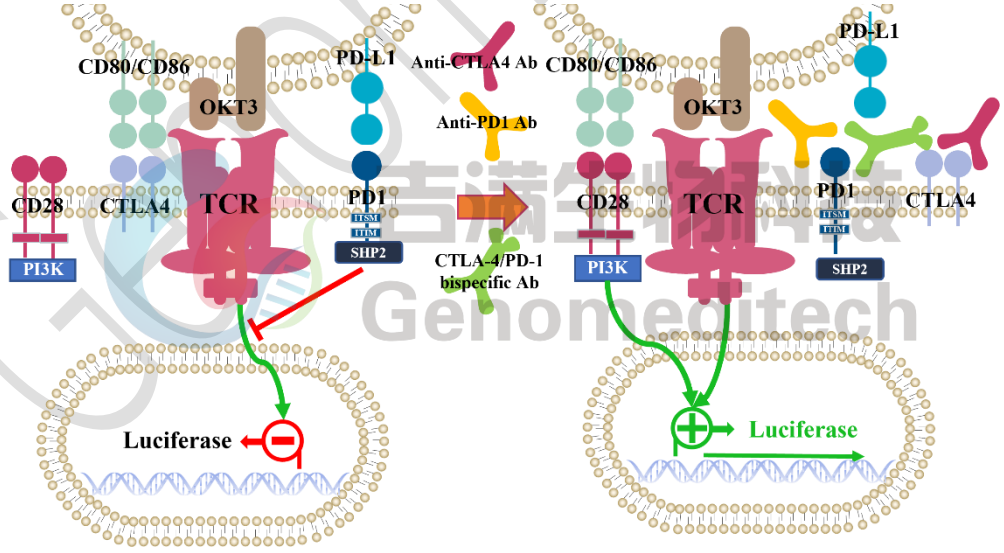
Version 3.3.1.250115

PD-1 is an immunosuppressive receptor on activated T and B cells that regulates immune responses by inhibiting TCR signaling through interaction with PD-L1/PD-L2. Blocking PD-1/PD-L1 with antibodies shows promise in cancer therapy.

CTLA-4 on Tregs suppresses immune responses by binding CD80/CD86 with higher affinity than CD28, inhibiting T cell activation. Antibodies targeting CTLA-4 have shown potential in cancer treatment.

Clinical data has shown that combining CTLA-4 and PD-1 antibodies or using bispecific CTLA-4/PD-1 antibodies results in better therapeutic effects than using either CTLA-4 or PD-1 antibodies alone.

H_CTLA4 PD-1 Reporter Cell Line is a clonal stable Jurkat cell line constructed using lentiviral technology, constitutive expression of the CTLA4 and PD1 gene, endogenously expression of the TCR-CD3 complex and CD28 gene, along with signal-dependent expression of a luciferase reporter gene. When T cells are stimulated by TCR (T-cell receptor) and CD80 binds to CD28, leading to the expression of luciferase. The PDL1 binds to PD1 or CTLA4 competes with CD28 for CD80, blockade the expression of luciferase. Blockade antibodies can block this inhibitory signal transmission, restore the activation of T cells. The luciferase activity measurement indicates the activation level of the signaling pathway and can thus be used to evaluate the in vitro effects of drugs related to CTLA4 and PD1.



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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+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
Hygromycin	Genomeditech/GM-040403
Puromycin	Genomeditech/GM-040401
H_CD80 PDL1 aAPC CHO-K1 Cell Line	Genomeditech/GM-C30574
Anti-H_CTLA-4 hIgG1 Antibody(Ipilimumab)	Genomeditech/GM-27203AB
Anti-PD1 hIgG4 Antibody(Pembrolizumab)	Genomeditech/GM-52674AB
Anti-CTLA-4/PD-1 hIgG1 Bispecific Antibody(Cadonilimab)	Genomeditech/GM-60293AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

Figures

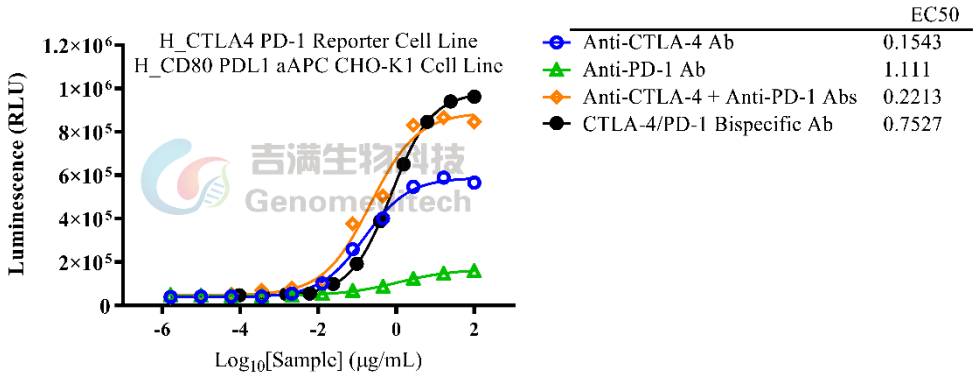


Figure 1 | Response to Ipilimumab, Pembrolizumab and Cadonilimab. The H_CD80 PDL1 aAPC CHO-K1 Cell Line (Cat. GM-C30574) was seeded at a density of 1.5E4 cells/well in a 96-well plate and incubated overnight. The next day, serial dilutions of Ipilimumab, Pembrolizumab, Ipilimumab+Pembrolizumab, and Cadonilimab, along with 1E5 cells/well of the H_CTLA4 PD-1 Reporter Cell Line (Cat. GM-C26486), were added to the pre-seeded cells. The mixture was incubated for 7 hours. Firefly luciferase activity was then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are presented as drug mass concentration.

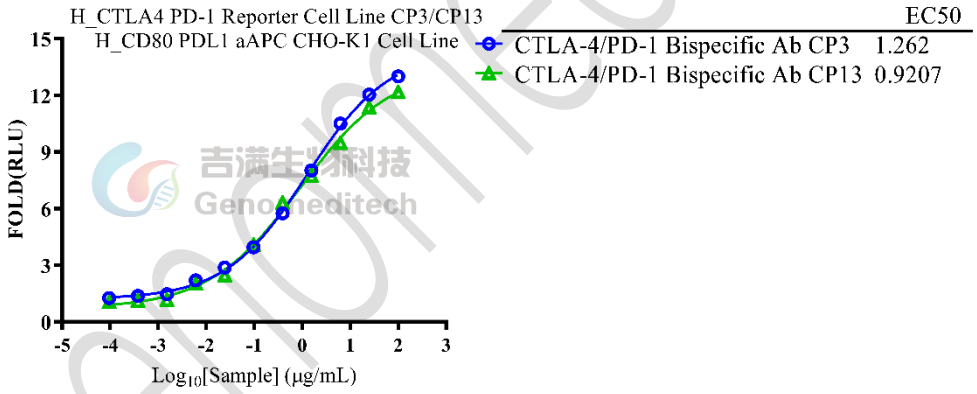
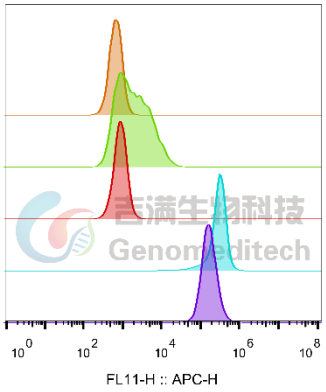


Figure 2 | The passage stability of response to Anti-CTLA-4/PD-1 hIgG1 Antibody. The H_CD80 PDL1 aAPC CHO-K1 Cell Line (Cat. GM-C30574) was seeded at a density of 1.5E4 cells/well in a 96-well plate and incubated overnight. The next day, serial dilutions of Anti-CTLA-4/PD-1 hIgG1 Antibody (Cadonilimab) (Cat. GM-60293AB), along with 1E5 cells/well of the CP3 and CP13 passage of H_CTLA4 PD-1 Reporter Cell Line (Cat. GM-C26486), were added to the pre-seeded cells. The mixture was incubated for 7 hours. Firefly luciferase activity was then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are presented as drug mass concentration.



SampleID	Geometric Mean : FL11-H
Cell anti-CTLA4+APC-2nd Ab	682
Cell anti-PD-1+APC-2nd Ab	1902
H_CTLA4 PD-1 Reporter Cell H_IgG+APC-2nd Ab	898
H_CTLA4 PD-1 Reporter Cell anti-CTLA4+APC-2nd Ab	274270
H_CTLA4 PD-1 Reporter Cell anti-PD-1+APC-2nd Ab	161413

Figure 3 | H_CTLA4 PD-1 Reporter Cell Line (Cat. GM-C26486) was determined by flow cytometry using Anti-H_CTLA-4 hIgG1 Antibody(Ipilimumab) (Cat. GM-27203AB) and 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.

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+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

CTLA4:CD80:CD86	
H_CD80 aAPC CHO-K1 Cell Line	H_CD80 PDL1 aAPC CHO-K1 Cell Line
H_CTLA4 Reporter Jurkat Cell Line	Canine_CTLA4 CHO-K1 Cell Line
Cynomolgus_CTLA4 HEK-293 Cell Line	H_CTLA4 CHO-K1 Cell Line
H_CTLA4 HEK-293 Cell Line	H_CTLA4 Jurkat Cell Line
Anti-CTLA4 hIgG1 Reference Antibody (Ipibio)	Anti-CTLA-4/PD-1 hIgG1 Bispecific Antibody(Cadonilimab)
Anti-H_CD80 hIgG1 Antibody(Galiximab)	Anti-H_CTLA-4 hIgG1 Antibody(Ipilimumab)
Anti-mouse CTLA4 mIgG2b Antibody(9D9)	Anti-mouse CTLA4 Syrian Hamster IgG2 Antibody(9H10)
Biotinylated Mouse CTLA4 Protein; His-Avi Tag	Mouse CTLA4 Protein; His Tag

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