Genomeditech (Shanghai) Co.,Ltd.
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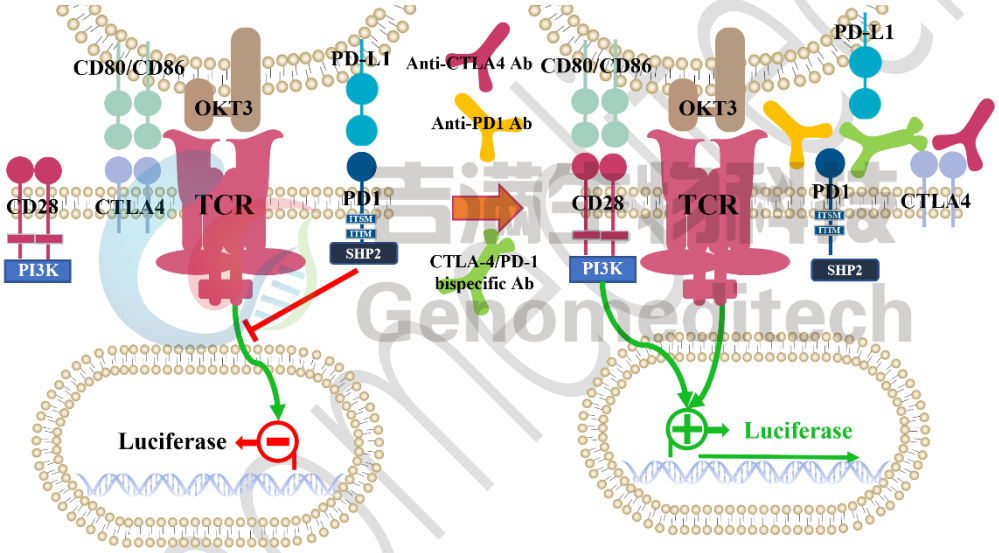
Product Sheet

H_CD80 PDL1 aAPC CHO-K1 Cell Line

Catalog number: GM-C30574

Version 3.3.1.250116

H_CD80 PDL1 aAPC CHO-K1 Cell Line is a clonal stable cell line that constitutively expresses OKT3, human PDL1 and human CD80 gene. The cell line is co-cultured with the H_CTLA4 PD-1 Reporter Cell Line (GM-C26486). The binding of OKT3 to TCR and CD80 to CD28 jointly activates T cell signaling. PD-L1 binds to PD-1, or CTLA-4 competes with CD28 for CD80, thereby blocking the expression of luciferase. Blockade antibodies can block this inhibitory signal transmission and 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	F12K+10% FBS+1% P.S
Growth medium	F12K+10% FBS+1% P.S+4 μg/mL Blasticidin+100 μg/mL Hygromycin+4 μg/mL Puromycin
Note	None
Freezing Medium	90% FBS+10%DMSO
Growth properties	Adherent
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.
F12K	BOSTER/PYG0036
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_CTLA4 PD-1 Reporter Cell Line	Genomeditech/GM-C26486
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
Anti-H_CD80 hIgG1 Antibody(Galiximab)	Genomeditech/GM-46075AB
Anti-H_CD274(PDL1) hIgG1 Antibody(Atezolizumab)	Genomeditech/GM-31740AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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Figures

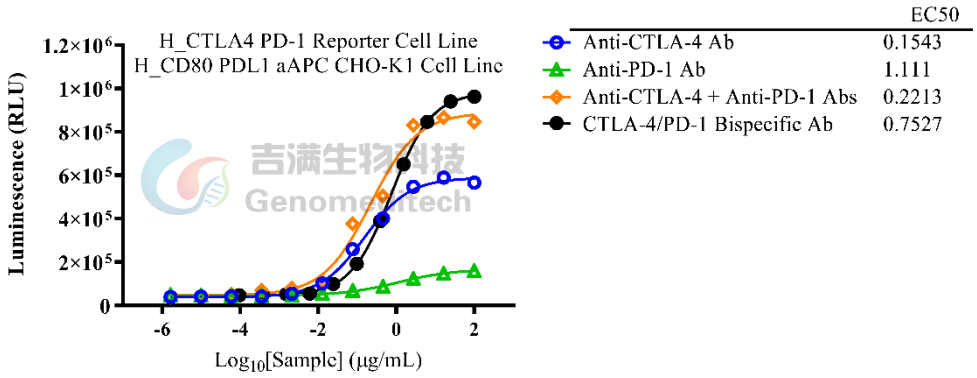
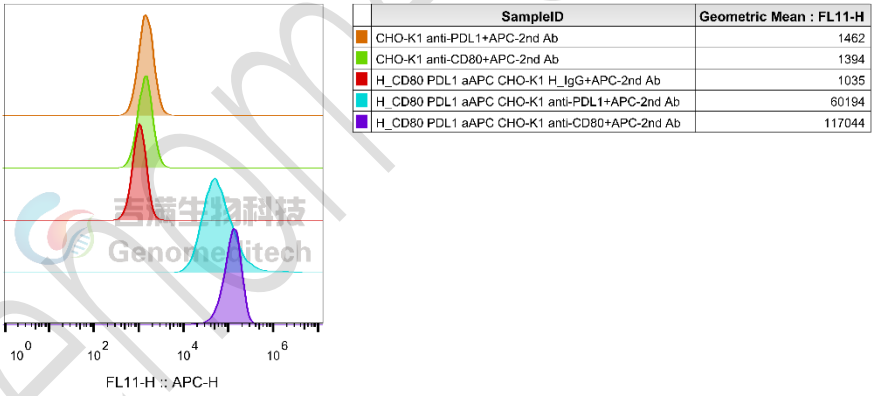


Figure 1 | Response to Ipilimumab, Pembrolizumab and Cadonilimab. The H_CD80 PDL1 aAPC CHO-K1 Cell Line (Cat. GM-C30574) was seeded at a density of 1.5E4 cells/well in a 96-well plate and incubated overnight. The next day, serial dilutions of Ipilimumab, Pembrolizumab, Ipilimumab+Pembrolizumab, and Cadonilimab, 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.



SampleID	Geometric Mean : FL11-H
CHO-K1 anti-PDL1+APC-2nd Ab	1462
CHO-K1 anti-CD80+APC-2nd Ab	1394
H_CD80 PDL1 aAPC CHO-K1 H_IgG+APC-2nd Ab	1035
H_CD80 PDL1 aAPC CHO-K1 anti-PDL1+APC-2nd Ab	60194
H_CD80 PDL1 aAPC CHO-K1 anti-CD80+APC-2nd Ab	117044

Figure 2 | H_CD80 PDL1 aAPC CHO-K1 Cell Line (Cat. GM-C30574) was determined by flow cytometry using Anti-H_CD80 hIgG1 Antibody(Galiximab) (Cat. GM-46075AB) and Anti-H_CD274(PDL1) hIgG1 Antibody(Atezolizumab) (Cat. GM-31740AB).

Cell Recovery

Recovery Medium: F12K+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 recovery medium. And dispense into appropriate culture dishes.
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: F12K+10% FBS+1% P.S+4 μg/mL Blasticidin+100 μg/mL Hygromycin+4 μg/mL Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

a) Remove and discard culture medium.
b) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
c) Add 1.0 mL of 0.25% (w/v) Trypsin-EDTA solution to dish and observe cells under an inverted microscope until cell layer is dispersed (usually within 2 to 3 minutes at 37°C).
d) Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
e) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
f) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
g) Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 - 1:5 is recommended

Medium Renewal: Every 2 to 3 days

Notes

a) After the stabilization of the cell condition, there will be fewer dead cells post-passage, the cell growth rate will tend to stabilize, cell morphology will become uniform, and the cells will appear robust.

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Related Products

CTLA4:CD80:CD86	
H_CD80 aAPC CHO-K1 Cell Line	H_CTLA4 PD-1 Reporter 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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