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# **Product Sheet**

## H\_CD80 aAPC CHO-K1 Cell Line

Catalog number: GM-C24688

Version 3.3.1.250116

The H\_CD80 aAPC CHO-K1 cell line is a clonal stable cell line that constitutively expresses the OKT3 and CD80 genes. This cell line is co-cultured with the H\_CD28 Reporter Jurkat Cell Line (GM-C25147). The binding of OKT3 to the TCR-CD3 complex and CD80 to CD28 jointly activates T cell signaling. By adding anti-CD28 antibodies, the interaction between CD80 and CD28 can be blocked, thereby inhibiting T cell signaling. It can be used to evaluate the in vitro effects of CD28 antagonist-like drugs.

In addition, this cell line can also be co-cultured with the H\_CTLA4 Reporter Jurkat Cell Line. The binding of OKT3 to the TCR-CD3 complex and CD80 to CD28 jointly activates T cell signaling; however, CTLA4 competes with CD28 for binding to CD80, thereby blocking the expression of luciferase. Blocking antibodies can inhibit this suppressive signaling pathway and restore T cell activation. The measurement of luciferase activity can be used to evaluate the in vitro effects of CTLA4 antagonist-like drugs.



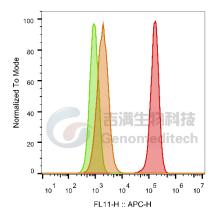
## Specifications

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	F12K+10% FBS+1% P.S		
Growth medium	F12K+10% FBS+1% P.S+4 µg/mL Blasticidin+4 µg/mL Puromycin		
Note	None		
Freezing Medium	90% FBS+10% DMSO		
Growth properties	Adherent		
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			
Reagent	Manufacturer/Catalogue No.		

Reagent	Manufacturer/Catalogue No.		
F12K	BOSTER/PYG0036		
Fetal Bovine Serum	Cegrogen biotech/A0500-3010		
Pen/Strep	Thermo/15140-122		
Blasticidin	Genomeditech/GM-040404		
Puromycin	Genomeditech/GM-040401		
Anti-H_CD80 hIgG1 Antibody(Galiximab)	Genomeditech/GM-46075AB		



#### Figures



SampleID	Geometric Mean : FL11-H
CHO-K1 anti-H_CD80+APC-2nd Ab	1916
H_CD80 aAPC CHO-K1 H_IgG+APC-2nd Ab	926
H_CD80 aAPC CHO-K1 anti-H_CD80+APC-2nd Ab	163459

Figure 1 | H\_CD80 aAPC CHO-K1 Cell Line (Cat. GM-C24688) was determined by flow cytometry using Anti-H\_CD80 hIgG1 Antibody(Galiximab) (Cat. GM-46075AB).

#### **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  $-70^{\circ}$ 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 recovery medium. And dispense into appropriate culture dishes.
- 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.
- 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.

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#### Cell passage

Growth medium: F12K+10% FBS+1% P.S+4 µg/mL Blasticidin+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).
- 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.

#### **Related Products**

CTLA4:CD80:CD86		
H_CD80 PDL1 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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