Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_CD47 PDL1 MC38(mouse_PDL1 KO) Cell Line

Catalog number: GM-C22137

Version 3.3.1.250115

Description	H_CD47 PDL1 MC38(mouse_PDL1 KO) Cell Line is a clonal stable MC38 cell line that continuously expresses human CD47 and human PDL1 genes. It is constructed using lentiviral technology, based on the knockout of mouse PDL1.
Quantity	5E6 Cells per vial,1 mL
Product Format	3 vials of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt

Target	Human_CD47 & Human_PDL1
Gene ID/Uniprot ID	Q08722-1 & NP_054862.1
Host Cell	MC38(mouse_PDL1 KO)

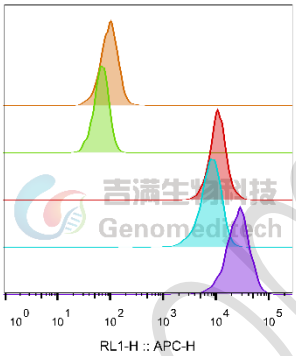
Recovery Medium	DMEM+10% FBS+1% P.S
Growth medium	DMEM+10% FBS+1% P.S+2 μg/mL Blasticidin+200 μg/mL G418+200 μg/mL Hygromycin+2.5 μg/mL Puromycin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Adherent
Growth Conditions	37°C, 5% CO_2

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

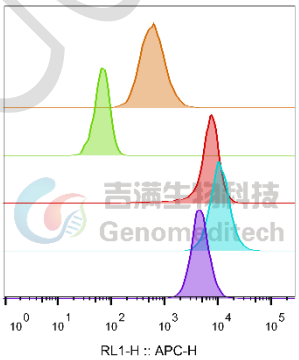
Reagent	Manufacturer/Catalogue No.
DMEM	VivaCell/C3110-0500
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
G418	Genomeditech/GM-040402
Hygromycin	Genomeditech/GM-040403
Puromycin	Genomeditech/GM-040401
Anti-CD47 hIgG4 Antibody(5F9)	Genomeditech/GM-27657AB
Anti-H_CD274(PDL1) hIgG1 Antibody(Atezolizumab)	Genomeditech/GM-31740AB

Figures



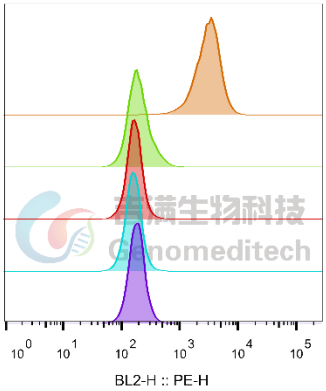
SampleID	Geometric Mean : RL1-H
MC38 anti-H_CD47+APC-2nd Ab	95.3
H_CD47 H_PDL1 MC38(Mouse_PDL1 KO) H_IgG+APC-2nd Ab	67.2
H_CD47 H_PDL1 MC38(Mouse_PDL1 KO) anti-H_CD47+APC-2nd Ab #1	10521
H_CD47 H_PDL1 MC38(Mouse_PDL1 KO) anti-H_CD47+APC-2nd Ab #2	7372
H_CD47 H_PDL1 MC38(Mouse_PDL1 KO) anti-H_CD47+APC-2nd Ab #9	24235

Figure 1 | H_CD47 PDL1 MC38(mouse_PDL1 KO) Cell Line (Cat. GM-C22137) was determined by flow cytometry using Anti-CD47 hIgG4 Antibody(5F9) (Cat. GM-27657AB).



SampleID	Geometric Mean : RL1-H
MC38 anti-PDL1+APC-2nd Ab	597
H_CD47 H_PDL1 MC38(Mouse_PDL1 KO) H_IgG+APC-2nd Ab	67.2
H_CD47 H_PDL1 MC38(Mouse_PDL1 KO) anti-PDL1+APC-2nd Ab #1	6268
H_CD47 H_PDL1 MC38(Mouse_PDL1 KO) anti-PDL1+APC-2nd Ab #2	10747
H_CD47 H_PDL1 MC38(Mouse_PDL1 KO) anti-PDL1+APC-2nd Ab #9	4565

Figure 2 | H_CD47 PDL1 MC38(mouse_PDL1 KO) Cell Line (Cat. GM-C22137) was determined by flow cytometry using Anti-H_CD274(PDL1) hIgG1 Antibody(Atezolizumab) (Cat. GM-31740AB).



SampleID	Geometric Mean : BL2-H
MC38 PE-anti-M_PDL1	2902
MC38(Mouse_PDL1 KO) H_PDL1 PE-R_IgG	197
MC38(Mouse_PDL1 KO) H_PDL1 PE-anti-M_PDL1 #1	167
MC38(Mouse_PDL1 KO) H_PDL1 PE-anti-M_PDL1 #2	160
MC38(Mouse_PDL1 KO) H_PDL1 PE-anti-M_PDL1 #9	183

Figure 3 | H_CD47 PDL1 MC38(mouse_PDL1 KO) Cell Line (Cat. GM-C22137) was determined by flow cytometry using PE anti-mouse CD274 (B7-H1, PD-L1) Antibody (BioLegend/124307).

Figure 4 | The Sanger sequencing of the H_CD47 PDL1 MC38(mouse_PDL1 KO) Cell Line (Cat. GM-C22137) showed successful knockout of mouse PDL1.

Cell Recovery

Recovery Medium: DMEM+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).

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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: DMEM+10% FBS+1% P.S+2 μg/mL Blasticidin+200 μg/mL G418+200 μg/mL Hygromycin+2.5 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 30 to 60 seconds 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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Sequence

CD47 Q08722-1

MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFKGRDIYTFDGA
LNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELTREGETIIELKYRVVSWFSPNENILI
VIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTGILIL
LHYYVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKT
IQPPRKAVEEPLNAFKESKGMMNDE*

CD274(PD-L1) NP_054862.1

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEE
DLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILV
VDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET*

Related Products

CD47:SIRPα	
H_SIRPα Blockade Reporter Cell Line	H_SIRPα Reporter Jurkat Cell Line
Cynomolgus_CD47 CHO-K1 Cell Line	H_CD47 aAPC CHO-K1 Cell Line
H_CD47 CHO-K1 cell line	H_CD47 MC38 Cell Line
H_SIRPA(SIRPα) CHO-K1 Cell Line	Mouse_CD47 CHO-K1 Cell Line
Anti-CD47 hIgG4 Antibody(5F9)	Anti-mouse SIRPA mIgG1 Antibody(p84)
Anti-mouse SIRPA RlgG1 Antibody(p84)	
In Vivo MAb Isotype Controls	
Human IgG1 Isotype Control(Anti-HEL)	Human IgG1 Isotype Control(Anti-MOPC-21)
Human IgG1 Isotype Control(Anti-RSV)	Human IgG1(LALA) Isotype Control(Anti-HEL)
Human IgG1(LALAPG) Isotype Control(Anti-HEL)	Human IgG1(N297A) Isotype Control(Anti-HEL)
Human IgG4(S228P) Isotype Control(Anti-HEL)	Mouse IgG1 Isotype Control(Anti-HEL)
Mouse IgG2a Isotype Control(Anti-HEL)	Mouse IgG2a Isotype Control(Anti-RSV)
Mouse IgG2a(D265A) Isotype Control(Anti-HEL)	

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