Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_NLRP3 KO THP1 Cell Line

Catalog number: GM-C39958

Version 3.3.1.260112

Description	H_NLRP3 KO THP1 Cell Line is a clonal stable cell line derived from THP1 cells with a knockout of human NLRP3.
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
Target	Human_NLRP3
Gene ID/Uniprot ID	/
Host Cell	THP1
Recovery Medium	RPMI 1640(ATCC)+10% FBS+1% P.S
Growth medium	RPMI 1640(ATCC)+10% FBS+1% P.S+0.05 mM β-Me+2 μg/mL Blasticidin
Note	Hard to revive; culture for about 2 weeks after revival.
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.

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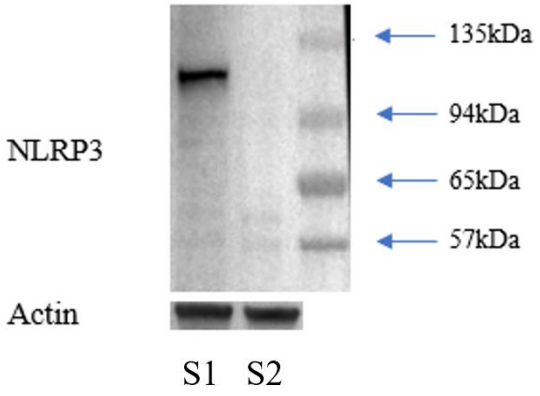
Materials

Reagent	Manufacturer/Catalogue No.
RPMI 1640(ATCC)	ATCC/30-2001
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
2-Mercaptoethanol(β-Me)	gibco/21985-023
Blasticidin	Genomeditech/GM-040404
NLRP3 (D4D8T) Rabbit Monoclonal Antibody	Cell Signaling/15101

Figures



S1:Con ; S2:H_NLRP3 KO THP1 Cell Line

Figure 1 | The protein expression levels of H_NLRP3 in the H_NLRP3 KO THP1 Cell Line(Cat. GM-C39958) were determined by Western blotting (WB).

Figure 2 | The Sanger sequencing of the H_NLRP3 KO THP1 Cell Line showed successful knockout of NLRP3.

Cell Recovery

Recovery Medium: RPMI 1640(ATCC)+10% FBS+1% P.S

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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 an appropriate culture flask and initially place the flask in an upright position after thawing.
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(ATCC)+10% FBS+1% P.S+0.05 mM β-Me+2 μg/mL Blasticidin

During the first three passages after cell thawing, use the recovery medium. Once the cell status stabilizes, switch to growth medium containing antibiotics.

a) When the cell density reaches 8E5 cells/mL, subculture the cells. Do not allow the cell density to exceed 1E6 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 8E5 viable cells/mL.

Medium Renewal: Every 2 to 3 days

Notes

a) Cells should be cultured in recovery medium for the first three passages post-thawing. Within the first three passages, 20% Fetal Bovine Serum (FBS) should be used, and then reduced to 10% FBS. This cell line is density-dependent

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and should be passaged into T25 flasks at a density of 3 × 105 - 1 × 106 cells/mL, with a culture volume of approximately 3-8 mL.

b) The cells are sensitive to density, so it is crucial to maintain the density within the appropriate range during culture and passage. They should not be used for detection or cryopreservation until the doubling rate stabilizes, typically after 7-10 days.

c) β-Mercaptoethanol must be added to the culture medium; omitting it may negatively impact cell condition. These cells are challenging to recover, with a post-thaw viability of approximately 60-80%, and require 5-7 days of culture to resume normal growth.

d) FBS should be heated at 56°C for 30 minutes to inactivate complements and certain viruses, without significantly affecting the activity of most growth factors and cytokines.

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