Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_SIRPα Blockade Reporter Cell Line

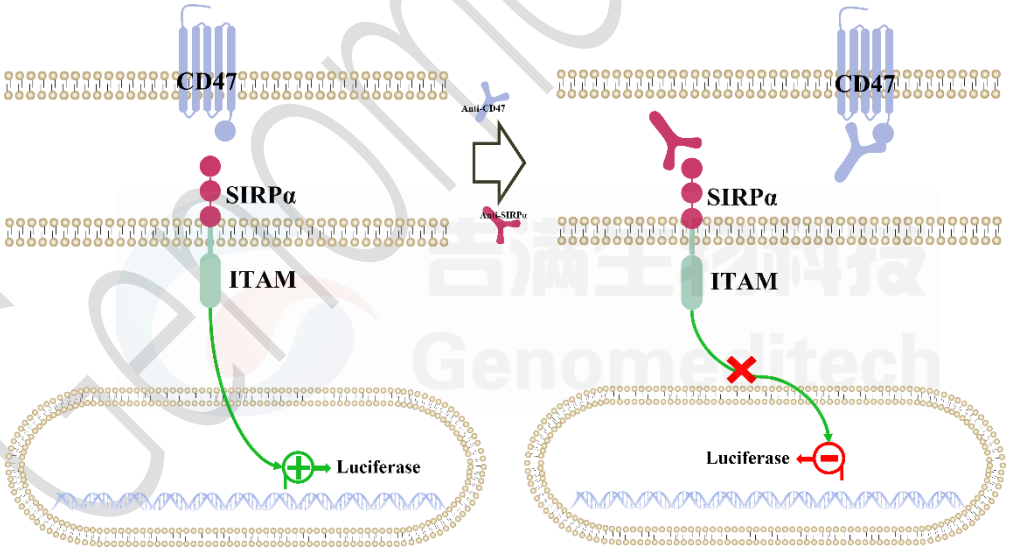
Catalog number: GM-C23900

Version 3.3.1.241218

SIRPα (Signal Regulatory Protein Alpha) is a cell surface protein in the immunoglobulin superfamily, mainly expressed on immune cells like macrophages and dendritic cells. It regulates immune responses by binding to its ligand CD47, primarily inhibiting macrophage phagocytosis, which is essential for immune surveillance and self-tolerance.

In signaling pathways, SIRPα activates downstream mechanisms through CD47 binding, inhibiting Src family tyrosine kinases. This interaction leads to the phosphorylation of SIRPα's intracellular domain, reducing macrophage activation and phagocytosis. SIRPα also affects immune cell function and tumor microenvironment formation by regulating cytokine release and intercellular interactions, making it important for research on tumor immune evasion and autoimmune diseases.

H_SIRPα Blockade Reporter Cell Line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of the SIRPα chimeric receptor gene, along with signal-dependent expression of a luciferase reporter gene. When CD47 binds to SIRPα, it activates downstream signaling pathways, leading to the expression of luciferase. Blockade antibodies can inhibit this signal transmission. 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 SIRPα.



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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+400 μg/mL G418+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
G418	Genomeditech/GM-040402
Hygromycin	Genomeditech/GM-040403
Puromycin	Genomeditech/GM-040401
H_CD47 CHO-K1 cell line	Genomeditech/GM-C09241
Anti-CD47 hIgG4 Antibody(5F9)	Genomeditech/GM-27657AB
Anti-H_SIRPα hIgG1 Antibody	Genomeditech/GM-46164AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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Figures

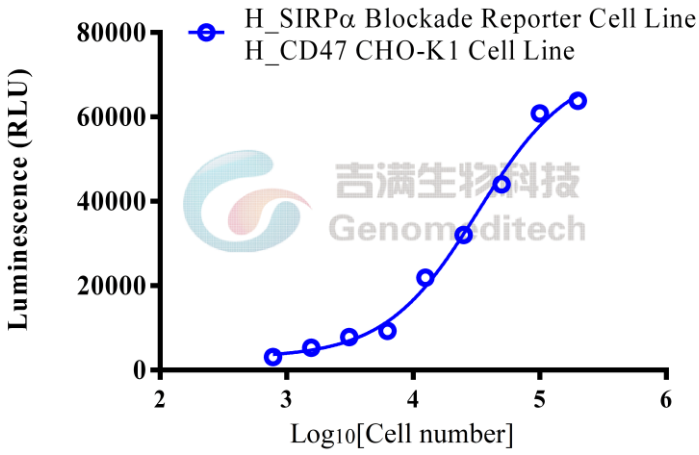

Figure 1 | Response to H_CD47 CHO-K1 cell line. H_SIRPα Blockade Reporter Cell Line (Cat. GM-C23900) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of H_CD47 CHO-K1 cell line (Cat. GM-C09241) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503).

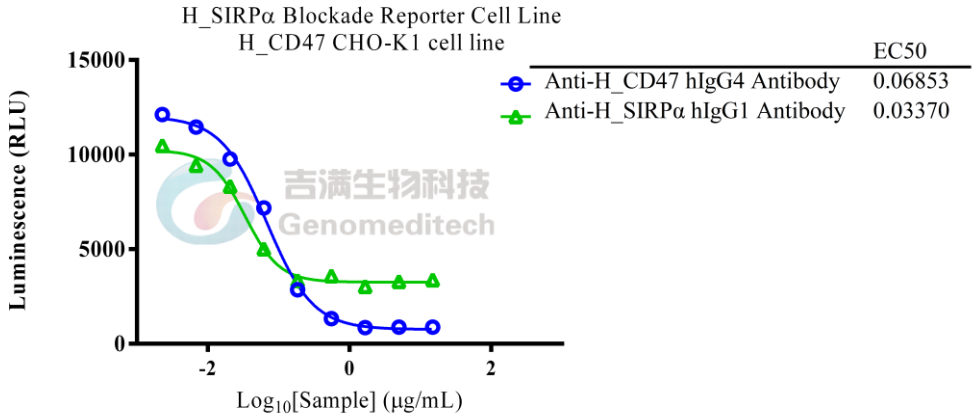

Figure 2 | Response to Anti-CD47 hIgG4 Antibody(5F9) and Anti-H_SIRPα hIgG1 Antibody. Serial dilutions of the Anti-H_SIRPα hIgG1 Antibody were incubated with 1.5E5 cells/well of the H_SIRPα Blockade Reporter Cell Line for 1 hour, then were added to the H_CD47 CHO-K1 cell line. Serial dilutions of theand the Anti-CD47 hIgG4 Antibody(5F9) were incubated of the H_CD47 CHO-K1 cell line for 1 hour, then the H_SIRPα Blockade Reporter Cell Line were added. The H_CD47 CHO-K1 cell line usage concentration is 1.5E4 cells/well. Subsequently, the coculture proceeded for an additional 15 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug mass concentration.

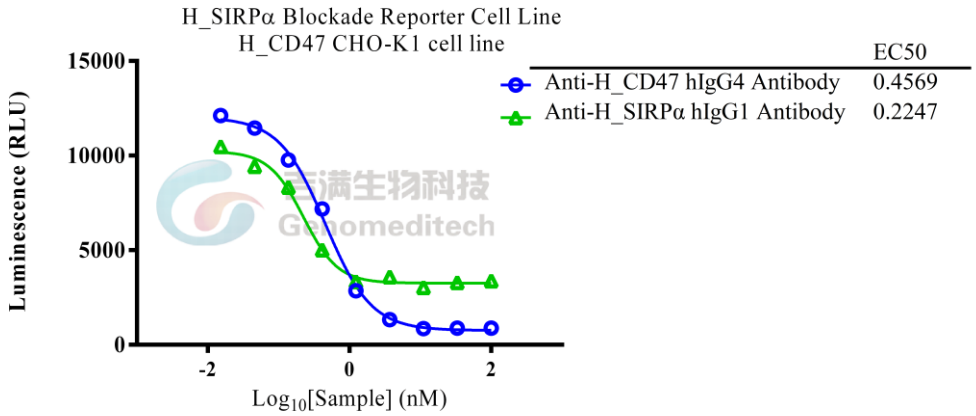

Figure 3 | Response to Anti-CD47 hIgG4 Antibody(5F9) and Anti-H_SIRPα hIgG1 Antibody. Serial dilutions of the Anti-H_SIRPα hIgG1 Antibody were incubated with 1.5E5 cells/well of the H_SIRPα Blockade Reporter Cell Line for 1 hour, then were added to the H_CD47 CHO-K1 cell line. Serial dilutions of theand the Anti-CD47 hIgG4 Antibody(5F9) were incubated of the H_CD47 CHO-K1 cell line for 1 hour, then the H_SIRPα Blockade Reporter Cell Line were added. The H_CD47 CHO-K1 cell line usage concentration is 1.5E4 cells/well. Subsequently, the coculture proceeded for an additional 15 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug molar concentration.

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+400 μg/mL G418+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

CD47:SIRPα	
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 LLC1 cell line	H_CD47 MC38 Cell Line
H_CD47 PDL1 MC38(mouse_PDL1 KO) 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)

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