Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

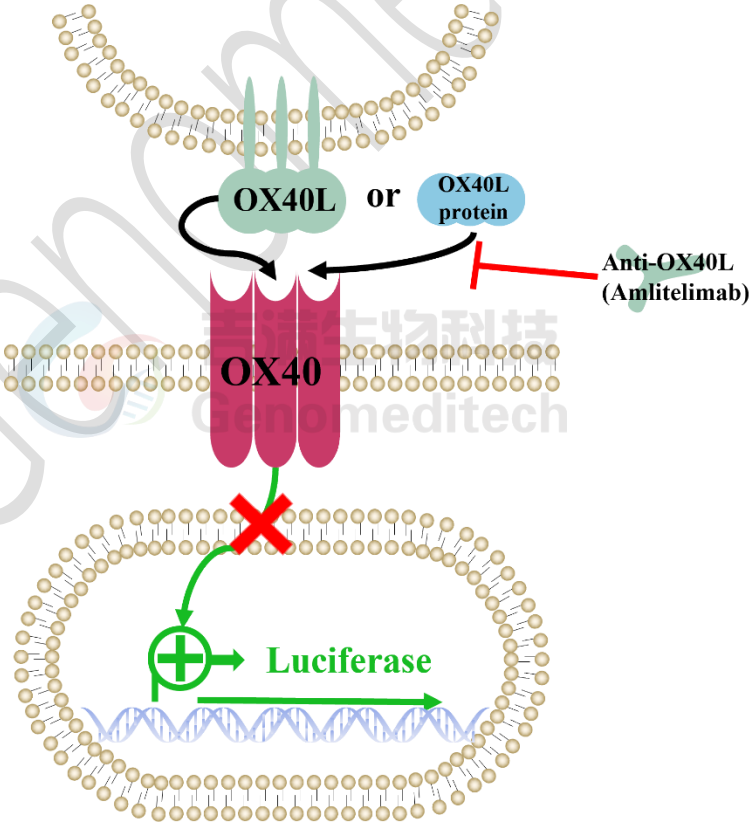
H_OX40 Reporter Cell Line

Catalog number: GM-C30855

Version 3.1.1.240913

OX40 is a cell membrane protein also known as CD134. It is an important costimulatory molecule that is mainly expressed on activated T cells and NK cells. OX40 signaling pathway in regulating immune response plays an important role in the process. When OX40 binds to its ligands, it activates multiple signaling pathways that promote T-cell proliferation, survival, and function. This signaling pathway is critical for regulating the activity of immune cells and the maintenance of immune responses. The OX40 signaling pathway is also considered to have potential applications in immunotherapy and immune regulation.

H_OX40 Reporter Cell Line is a clonal stable cell line constitutively expressing the OX40, a luciferase reporter gene. The addition of OX40L ligand protein agonists stimulates OX40L to bind OX40, activating downstream reporter genes and inducing luciferase expression. Blockade antibodies can block this signal transmission. The luciferase readout represents the activation level of the signaling pathway and can thus be used for evaluating the in vitro effects of neutralizing antibody of OX40.



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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+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.

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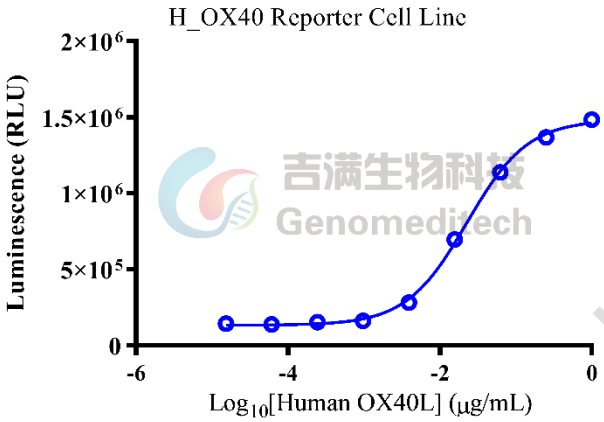
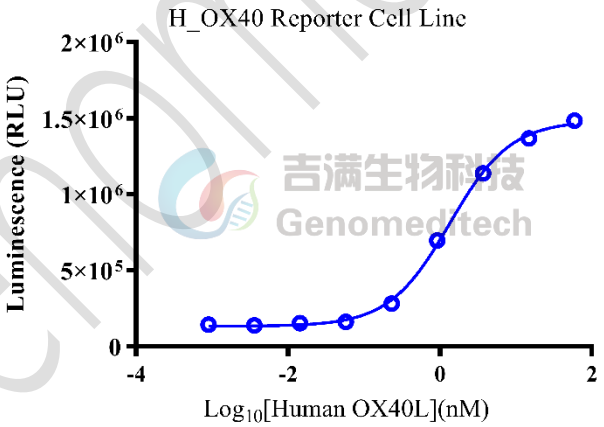
Figures



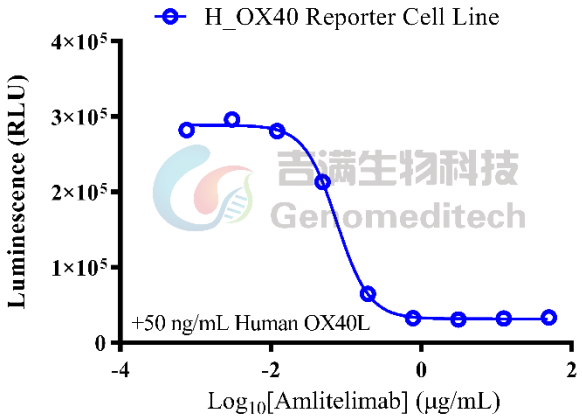
	H_OX40 Reporter Cell Line
EC50	0.02274

Figure 1 | Response to human OX40 ligand protein. The H_OX40 Reporter Cell Line (Genomeditech/GM-C30855) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Human OX40L Protein (Genomeditech/GM-83111RP) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Genomeditech/GM-040503C). The maximum induction fold was approximately [11.8]. Data are shown by drug mass concentration.



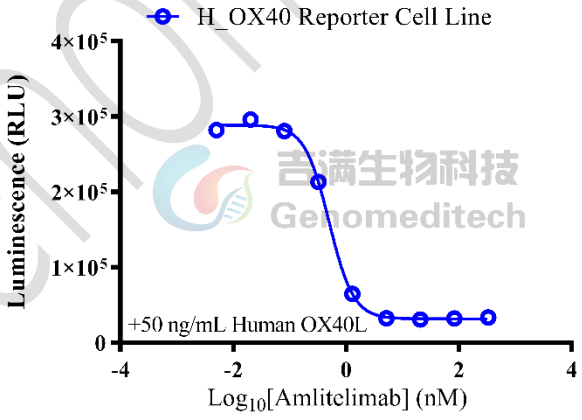
	H_OX40 Reporter Cell Line
EC50	1.345

Figure 2 | Response to human OX40 ligand protein. The H_OX40 Reporter Cell Line (Genomeditech/GM-C30855) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Human OX40L Protein (Genomeditech/GM-83111RP) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Genomeditech/GM-040503C). The maximum induction fold was approximately [11.8]. Data are shown by drug molar concentration.



	H_OX40 Reporter Cell Line
IC50	0.07545

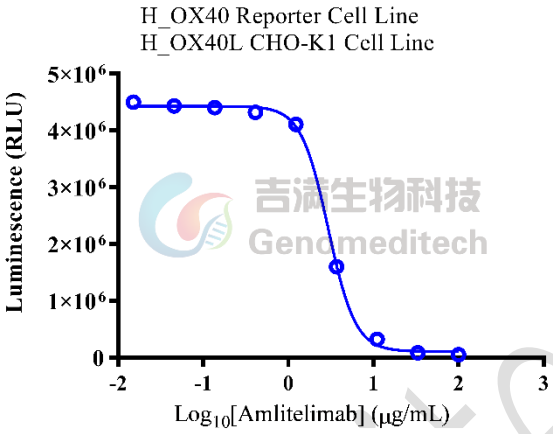
Figure 3 | Response to Anti-OX40L hIgG4 Antibody(Amlitelimab). Begin by preparing the H_OX40 Reporter Cell Line (Genomeditech/GM-C30855) at a density of 1E5 cells/well in a 96-well format. The serial dilutions of Anti-OX40L hIgG4 Antibody(Amlitelimab) (Genomeditech/GM-82533AB) were incubated with 50 ng/mL of Human OX40L Protein (Genomeditech/GM-83111RP) for 1 hour. After pre-incubation, the antibody-OX40L mixture was added to the H_OX40 Reporter Cell Line and incubated for 6 hours in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Genomeditech/GM-040503C). The results indicated a maximum blocking fold of approximately [8.9]. Data are shown by drug mass concentration.



	H_OX40 Reporter Cell Line
IC50	0.5030

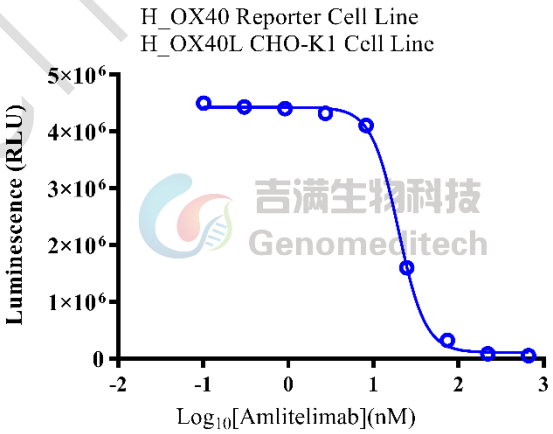
Figure 4 | Response to Anti-OX40L hIgG4 Antibody(Amlitelimab). Begin by preparing the H_OX40 Reporter Cell Line (Genomeditech/GM-C30855) at a density of 1E5 cells/well in a 96-well format. The serial dilutions of Anti-OX40L hIgG4 Antibody(Amlitelimab) (Genomeditech/ GM-82533AB) were incubated with 50 ng/mL of Human OX40L Protein (Genomeditech/GM-83111RP) for 1 hour. After pre-incubation, the antibody-OX40L mixture was added to the H_OX40 Reporter Cell Line and incubated for 6 hours in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). Firefly

luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Genomeditech/GM-040503C). The results indicated a maximum blocking fold of approximately [8.9]. Data are shown by drug molar concentration.



	H_OX40 Reporter Cell Line
IC50	2.958

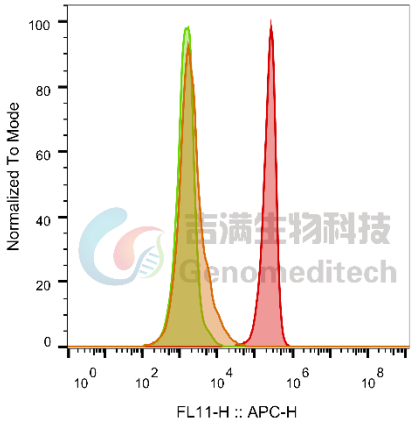
Figure 5 | Response to Anti-OX40L hIgG4 Antibody(Amlitelimab). Serial dilutions of the Anti-OX40L hIgG4 Antibody (Amlitelimab) was incubated with 1E4 cells/well of the H_OX40L CHO-K1 Cell Line (Genomeditech/GM-C35016) in a 96-well plate for 1 hour. Subsequently, the H_OX40 Reporter Cell Line (Genomeditech/GM-C30855) at a concentration of 1E5 cells/well was added, and the co-culture proceeded for an additional 6 hours in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Genomeditech/GM-040503C). The results indicated a maximum blocking fold of approximately [87.8]. Data are shown by drug mass concentration.



	H_OX40 Reporter Cell Line
IC50	19.72

Figure 6 | Response to Anti-OX40L hIgG4 Antibody(Amlitelimab). Serial dilutions of the Anti-OX40L hIgG4 Antibody (Amlitelimab) was incubated with 1E4 cells/well of the H_OX40L CHO-K1 Cell Line (Genomeditech/GM-C35016) in

a 96-well plate for 1 hour. Subsequently, the H_OX40 Reporter Cell Line (Genomeditech/GM-C30855) at a concentration of 1E5 cells/well was added, and the co-culture proceeded for an additional 6 hours in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Genomeditech/GM-040503C). The results indicated a maximum blocking fold of approximately [87.8]. Data are shown by drug molar concentration.



SampleID	Geometric Mean : FL11-H
Null anti-OX40+APC-2nd Ab	2011
H_OX40 Reporter H_IgG+APC-2nd Ab	1432
H_OX40 Reporter anti-OX40+APC-2nd Ab	230158

Figure 7 | H_OX40 Reporter Cell Line was determined by flow cytometry using Anti-H_OX40 hIgG2 Antibody(Ivuxolimab) (Genomeditech/GM-23373AB).

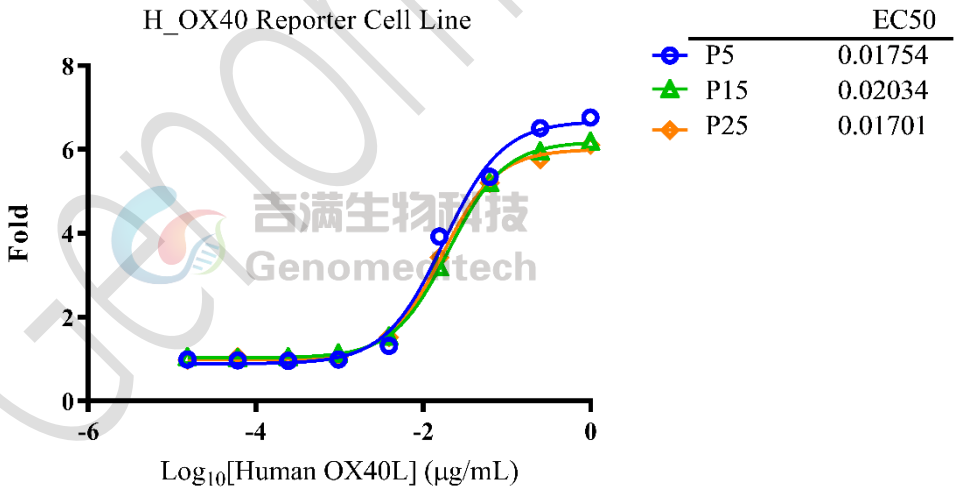


Figure 8 | Response to Human OX40L ligand protein. The passage 5,15 and 25 of H_OX40 Reporter Cell Line (Genomeditech/GM-C30855) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Human OX40L Protein (Genomeditech/GM-83111RP) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Genomeditech/GM-040503C). Data are shown by drug mass concentration.

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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+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.

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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.

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