Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_OX40 Reporter DDX35™ Cell Line

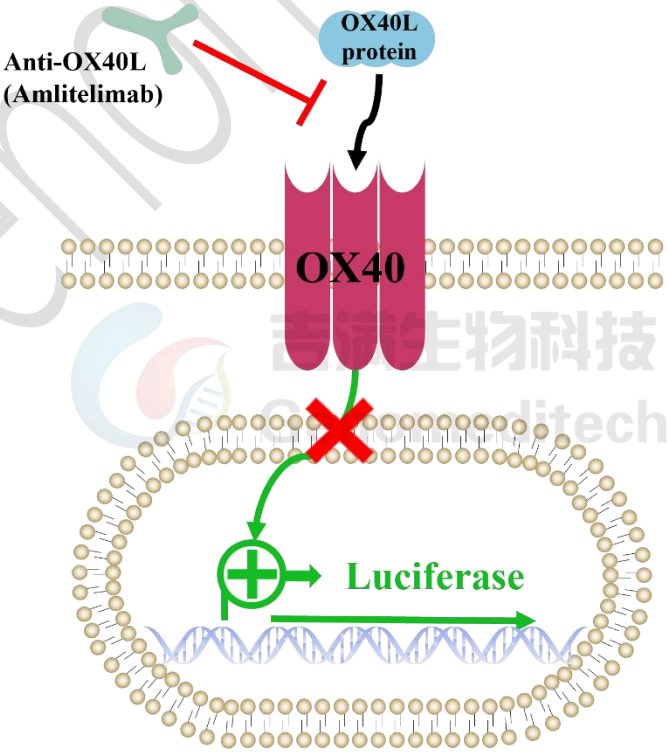
Catalog number: GM-C36735

Version 3.3.1.260422

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 DDX35™ Cell Line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of the OX40 gene, along with signal-dependent expression of a luciferase reporter gene. When OX40L binds to OX40, it activates downstream signaling pathways, leading to the expression of luciferase. The luciferase readout represents the activation level of the signaling pathway and can thus be used for evaluating the in vitro effects of related drugs of OX40.

H_OX40 Reporter DDX35™ Cell Line was obtained through extensive monoclonal screening and multiple rounds of monoclonal selection. It possesses high stability, high sensitivity, and high amplification properties, meeting the standards for customers' batch library construction and release experiments.



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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+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	gibco/C11875500BT
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
Human OX40L Protein; His Tag	Genomeditech/GM-83111RP
Anti-H_OX40 hIgG2 Antibody(Ivuxolimab)	Genomeditech/GM-23373AB
Anti-OX40L hIgG4 Antibody(Amlitelimab)	Genomeditech/GM-82533AB
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

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Figures

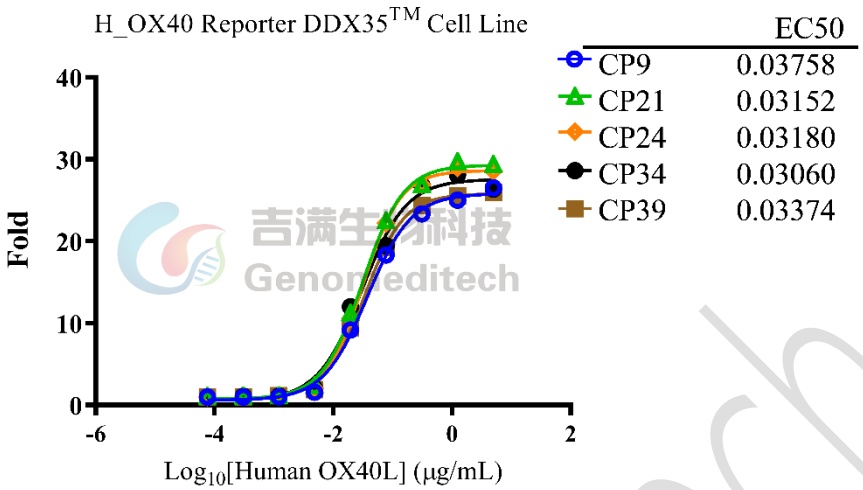


Figure 1 | The passage stability of response to Human OX40L Protein; His Tag. The passage 9,21,24,34 and 39 of H_OX40 Reporter DDX35™ Cell Line (Cat. GM-C36735) at a concentration of 5E4 cells/well (96-well format) was stimulated with serial dilutions of Human OX40L Protein (Cat. GM-83111RP) in assay buffer (RPMI 1640 + 10% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug mass concentration.

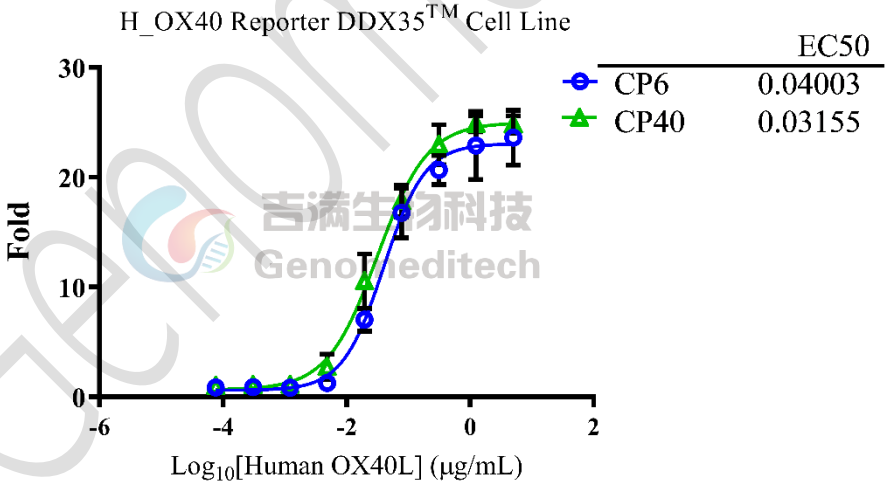
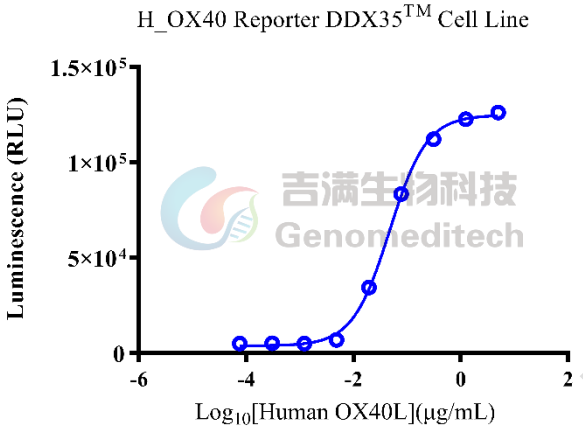
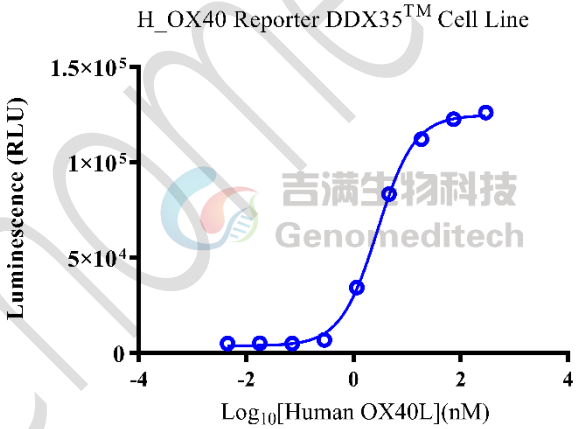


Figure 2 | The passage stability of response to Human OX40L Protein; His Tag. The passage 6 and 40 of H_OX40 Reporter DDX35™ Cell Line (Cat. GM-C36735) at a concentration of 5E4 cells/well (96-well format) was stimulated with serial dilutions of Human OX40L Protein (Cat. GM-83111RP) in assay buffer (RPMI 1640 + 10% FBS + 1% P.S) for 6 hours, in triplicate. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug mass concentration.



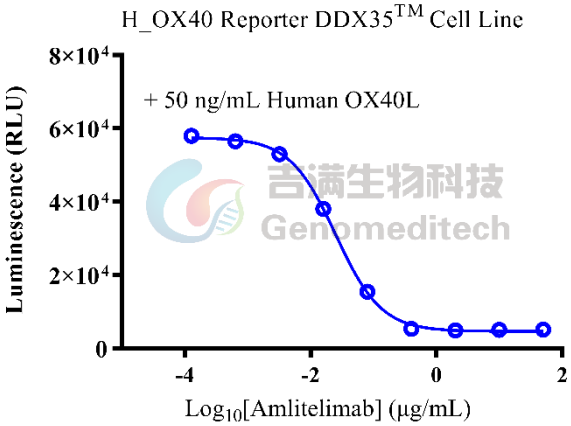
	H_OX40 Reporter DDX35TM
EC50	0.04758

Figure 3 | Response to Human OX40L Protein; His Tag. The H_OX40 Reporter DDX35™ Cell Line (Cat. GM-C36735) at a concentration of 5E4 cells/well (96-well format) was stimulated with serial dilutions of Human OX40L Protein (Cat. GM-83111RP) in assay buffer (RPMI 1640 + 10% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [21.7]. Data are shown by drug mass concentration.



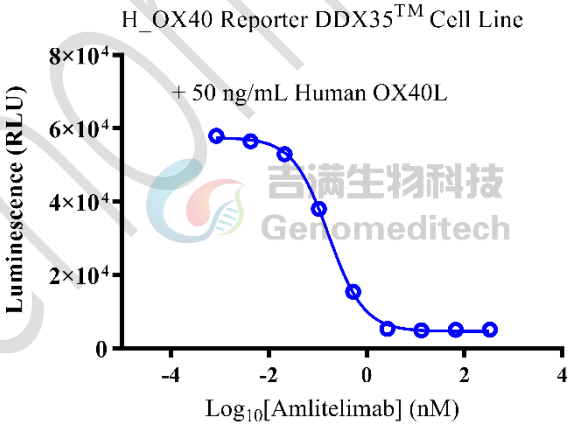
	H_OX40 Reporter DDX35TM
EC50	2.815

Figure 4 | Response to Human OX40L Protein; His Tag. The H_OX40 Reporter DDX35™ Cell Line (Cat. GM-C36735) at a concentration of 5E4 cells/well (96-well format) was stimulated with serial dilutions of Human OX40L Protein (Cat. GM-83111RP) in assay buffer (RPMI 1640 + 10% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech)). The maximum induction fold was approximately [21.7]. Data are shown by drug molar concentration.



	H_OX40 Reporter DDX35TM
IC50	0.02502

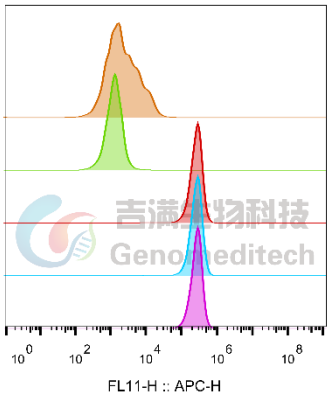
Figure 5 | Response to Anti-OX40L hIgG4 Antibody(Amlitelimab). Serial dilutions of Anti-OX40L hIgG4 Antibody(Amlitelimab)(Cat. GM-82533AB) were incubated with 5 ng/well of Human OX40L Protein (Cat. GM-83111PR) for 1 hour in assay buffer (RPMI 1640+10% FBS+1% P.S). After pre-incubation, add the mixture to the H_OX40 Reporter DDX35™ Cell Line (Cat. GM-C36735) at a density of 5E4 cells/well in a 96-well format, and incubate for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The results indicated maximum blocking folds of approximately [11.7]. Data are shown by drug mass concentration.



	H_OX40 Reporter DDX35TM
IC50	0.1668

Figure 6 | Response to Anti-OX40L hIgG4 Antibody(Amlitelimab). Serial dilutions of Anti-OX40L hIgG4 Antibody(Amlitelimab)(Cat. GM-82533AB) were incubated with 5 ng/well of Human OX40L Protein (Cat. GM-83111PR) for 1 hour in assay buffer (RPMI 1640+10% FBS+1% P.S). After pre-incubation, add the mixture to the H_OX40 Reporter DDX35™ Cell Line (Cat. GM-C36735) at a density of 5E4 cells/well in a 96-well format, and incubate for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit

(Genomeditech). The results indicated maximum blocking folds of approximately [11.7]. Data are shown by drug molar concentration.



SampleID	Geometric Mean : FL11-H
Null anti-OX40+APC-2nd	2123
H_OX40 Reporter DDX35 H_IgG+APC-2nd	1210
H_OX40 Reporter DDX35 anti-OX40+APC-2nd CP7	245982
H_OX40 Reporter DDX35 anti-OX40+APC-2nd CP30	247793
H_OX40 Reporter DDX35 anti-OX40+APC-2nd CP41	260391

Figure 7 | The passage stability of the H_OX40 Reporter DDX35™ Cell Line (Cat. GM-C36735) was determined by flow cytometry using Anti-H_OX40 hIgG2 Antibody(Ivuxolimab) (Cat. GM-23373AB).

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.

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

OX40:OX40L	
H_OX40 Reporter Cell Line	Cynomolgus_OX40L CHO-K1 Cell Line
H_OX40 CHO-K1 Cell Line	H_OX40L CHO-K1 Cell Line
H_OX40L HEK-293 Cell Line	Mouse_OX40L CHO-K1 Cell Line
Anti-H_OX40 hIgG2 Antibody(Ivuxolimab)	Anti-OX40L hIgG1 Reference Antibody(Oxebio)
Anti-OX40L hIgG4 Antibody(Amlitelimab)	Anti-OX40L hIgG4 Reference Antibody(Amlbio)
Biotinylated Human OX40L Protein; His-Avi Tag	Cynomolgus OX40 Protein; His Tag
Cynomolgus OX40L Protein; His Tag	Cynomolgus OX40L Protein; mFc Tag
Human OX40 Protein; His Tag	Human OX40L Protein; His Tag
Human OX40L Protein; mFc Tag	
IL-4/IL-13	

IL-4 Reporter Cell Line	IL-4/IL-13 Reporter 293 Cell Line
IL-4/IL-13 Reporter 293 DDX35TM Cell Line	Cynomolgus_IL4R CHO-K1 Cell Line
H_IL4R CHO-K1 Cell Line	H_IL4R CHO-K1 Cell Line (Low Expression)
H_IL4R HEK-293 Cell Line	Mouse_IL4R CHO-K1 Cell Line
Anti-IL13 hIgG4 Reference Antibody (Lebribio)	Anti-IL-4R hIgG1 Antibody(12B5)
Anti-IL4R hIgG4 Antibody(Dupilumab)	Anti-IL4R hIgG4 Reference Antibody (Dupbio)
Anti-IL-4R×IL31 hIgG4 Reference Antibody (PRO2198)	Anti-Mouse IL13 mIgG2a Antibody (BAK209B11)
Anti-Mouse IL-4RA mIgG1 Antibody	
Biotinylated Human IL-4R alpha Protein; Avi-His Tag	Cynomolgus IL-13 Protein; His Tag
Cynomolgus IL-4 Protein; His Tag	Cynomolgus IL-4R alpha Protein; His Tag
Human IL-13 Protein; His Tag	Human IL-13RA1 Protein; His Tag
Human IL-4 Protein; His Tag	Human IL-4R alpha Protein; hFc Tag
Human IL-4R alpha Protein; His Tag	Human IL-4R alpha Protein; mFc Tag
Mouse IL-13 Protein; His Tag	Mouse IL-4R alpha Protein; His Tag
Rat IL-4R alpha Protein; His Tag	
IL-31	
Cynomolgus_IL-31RA OSMR Reporter Baf3 Cell Line	H_IL-31 Reporter Cell Line
H_IL-31 Reporter DDX35TM Cell Line	Cynomolgus_IL31RA CHO-K1 Cell Line
H_IL31RA CHO-K1 Cell Line	H_IL31RA HEK-293 Cell Line
H_IL-31RA OSMR Baf3 Cell Line	H_IL-31RA OSMR CHO-K1 Cell Line
Anti-IL31 hIgG1 Antibody(mAb33)	Anti-IL-31 hIgG4 Reference Antibody (BMS-981164)
Anti-IL31RA hIgG1 Antibody(NA633)	Anti-IL31RA hIgG2 Antibody(Nemolizumab)
Anti-IL31RA hIgG2 Reference Antibody (Nemobio)	Anti-IL-4R×IL31 hIgG4 Reference Antibody (PRO2198)
Anti-OSMR hIgG4 Antibody(Vixarelimab)	Anti-OSMR hIgG4 Reference Antibody (Vixabio)
Cynomolgus IL-31 Protein; His Tag	Human IL-31 Protein; His Tag
Human IL-31RA Protein; hFc Tag	
MRGPRX2	
H_MRGPRX2 Gq Reporter CHO-K1 Cell Line	H_MRGPRX2 Gqi5 Reporter CHO-K1 Cell Line
Tango-H_MRGPRX2 CHO-K1 Cell Line	Cynomolgus_MRGPRX2 CHO-K1 Cell Line
Cynomolgus_MRGPRX2 HEK-293 Cell Line	Flag-Mouse_Mrgprb2 CHO-K1 Cell Line
Flag-Rat_Mrgprb3 HEK-293 Cell Line	H_MRGPRX2 CHO-K1 Cell Line
H_MRGPRX2 HEK-293 Cell Line	H_MRGPRX2 HMC-1 Cell Line
H_MRGPRX2 RBL-2H3 Cell Line	

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