Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_IL-31 Reporter Cell Line

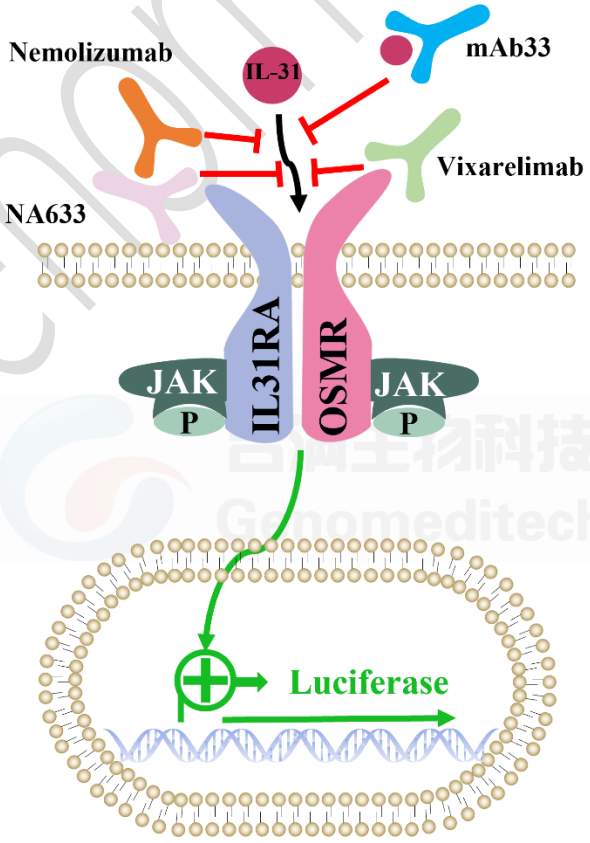
Catalog number: GM-C25989

Version 3.3.1.241219

Interleukin-31 (IL-31) is a novel cytokine belonging to the IL-6 cytokine family, primarily secreted by activated CD4+ T lymphocytes, especially Th2 cells, mast cells, macrophages, and dendritic cells. IL-31 regulates skin cell-mediated immunity by sensing itch in the nervous system, increases airway inflammation to modulate lung immunity, and influences gut immunity through microbial defense.

IL-31 signals through a heterodimer complex formed with IL31RA and OSMR. Its signaling pathway is closely associated with chronic itchy skin diseases such as atopic dermatitis. Monoclonal antibody drugs targeting IL-31 or its receptors can effectively reduce itching and sleep disturbances, improve skin lesions, and minimize the use of topical steroids.

H_IL-31 Reporter Cell Line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of the IL31RA and OSMR, along with signal-dependent expression of a luciferase reporter gene. When IL-31 binds to IL-31RA and OSMR heterodimer, 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 IL-31.



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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	DMEM+10% FBS+1% P.S
Growth medium	DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin+400 μg/mL G418+0.75 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	Gibco/C11995500BT
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
G418	Genomeditech/GM-040402
Puromycin	Genomeditech/GM-040401
Recombinant Human IL-31 Protein	Sino Biological/11557-H08H
Anti-IL31RA hIgG2 Antibody(Nemolizumab)	Genomeditech/GM-50871AB
Anti-IL31RA hIgG1 Antibody(NA633)	Genomeditech/GM-50880AB
Anti-OSMR hIgG4 Antibody(Vixarelimab)	Genomeditech/GM-50874AB
Anti-IL31 hIgG1 Antibody(mAb33)	Genomeditech/GM-50883AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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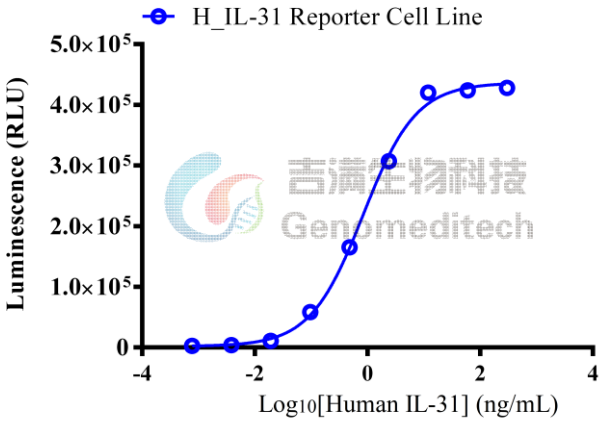
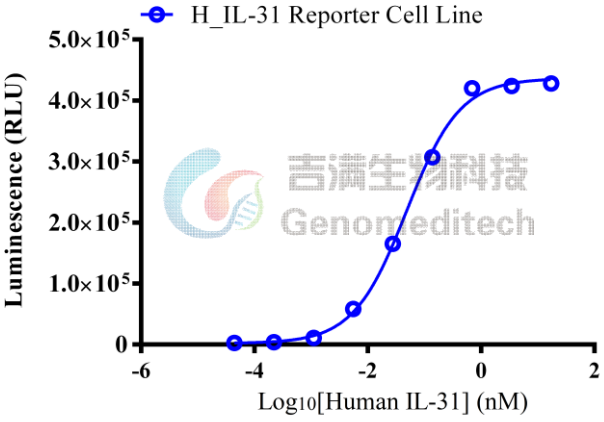
Figures



	H_IL-31 Reporter Cell Line
EC50	0.8408

Figure 1 | Response to Recombinant Human IL-31 Protein. H_IL-31 Reporter Cell Line (GM-C25989) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-31 Protein (Sino Biological/11557-H08H) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [152.4]. Data are shown by drug mass concentration.



	H_IL-31 Reporter Cell Line
EC50	0.04860

Figure 2 | Response to Recombinant Human IL-31 Protein. H_IL-31 Reporter Cell Line (GM-C25989) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-31 Protein (Sino Biological/11557-H08H) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [152.4]. Data are shown by drug molar concentration.

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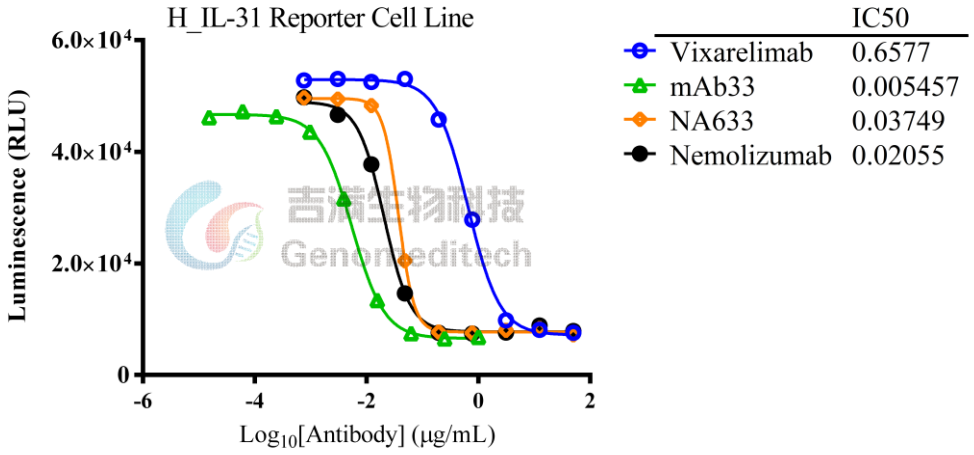

Figure 3 | Response to Nemolizumab, NA633, Vixarelimab and mAb33. Serial dilutions of the Nemolizumab, NA633, Vixarelimab were incubated with 1.5E4 cells/well of the H_IL-31 Reporter Cell Line (Cat. GM-C25989) for 1 hour, then the Recombinant Human IL-31 Protein was added. Serial dilutions of the mAb33 were incubated of the Recombinant Human IL-31 Protein for 1 hour, then were added to the H_IL-31 Reporter Cell Line. The protein usage concentration is 0.084 ng/well. Subsequently, the coculture proceeded for an additional 6 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.

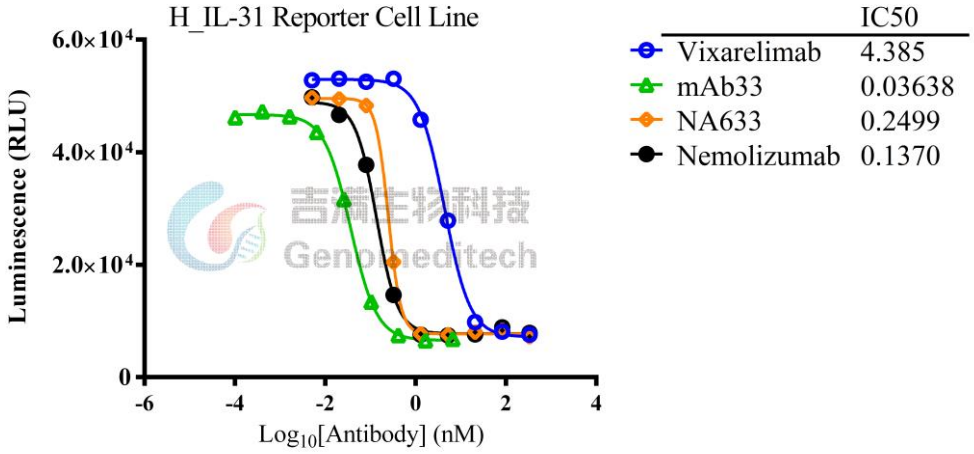

Figure 4 | Response to Nemolizumab, NA633, Vixarelimab and mAb33. Serial dilutions of the Nemolizumab, NA633, Vixarelimab were incubated with 1.5E4 cells/well of the H_IL-31 Reporter Cell Line (Cat. GM-C25989) for 1 hour, then the Recombinant Human IL-31 Protein was added. Serial dilutions of the mAb33 were incubated of the Recombinant Human IL-31 Protein for 1 hour, then were added to the H_IL-31 Reporter Cell Line. The protein usage concentration is 0.084 ng/well. Subsequently, the coculture proceeded for an additional 6 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.

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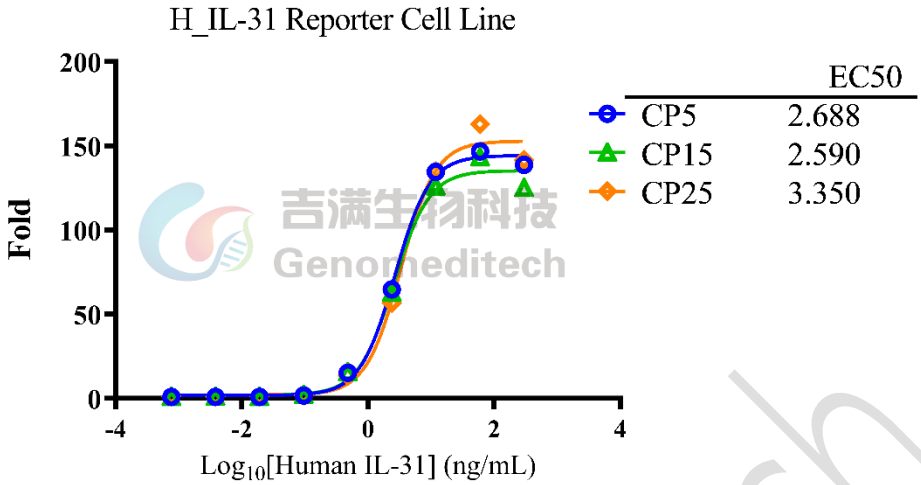


Figure 5 | The passage stability of response to Recombinant Human IL-31 Protein. The passage 5, 15, and 25 of H_IL-31 Reporter Cell Line (Cat. GM-C25989) at a concentration of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Recombinant Human IL-31 Protein (Sino Biological/11557-H08H) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug mass concentration.

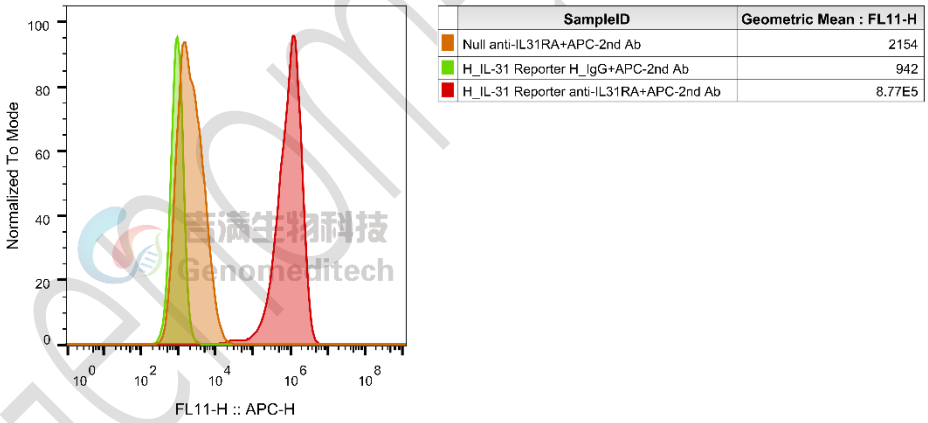
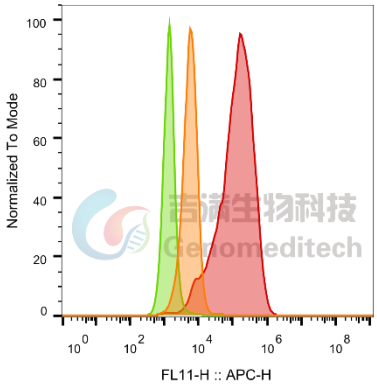


Figure 6 | H_IL-31 Reporter Cell Line (Cat. GM-C25989) was determined by flow cytometry using Anti-IL31RA hIgG2 Antibody(Nemolizumab) (Cat. GM-50871AB).



SampleID	Geometric Mean : FL11-H
Null anti-OSMR+APC-2nd Ab	5675
H_IL-31 Reporter H_IgG+APC-2nd Ab	1407
H_IL-31 Reporter anti-OSMR+APC-2nd Ab	116473

Figure 7 | H_IL-31 Reporter Cell Line (Cat. GM-C25989) was determined by flow cytometry using Anti-OSMR hIgG4 Antibody(Vixarelimab) (Cat. GM-50874AB).

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).

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.

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Cell passage

Growth medium: DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin+400 μg/mL G418+0.75 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) Subculturing is necessary when the cell density reaches 80%. It is recommended to perform subculturing at a ratio of 1:3 to 1:4 every 2-3 days. Ensure that the density does not exceed 80%, as overcrowding can lead to reduced viability due to compression.

b) Remove and discard culture medium.

c) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.

d) 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).

e) 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.

f) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.

g) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.

h) Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 - 1:4 is recommended

Medium Renewal: Every 2 to 3 days

Notes

a) Upon initial thawing, a higher number of dead cells is observed, which is a normal phenomenon. Significant improvement is seen after adaptation. Once the cells reach a stable state, the number of dead cells decreases after subculturing and the cell growth rate becomes stable.

b) Ensure that the cell density does not exceed 80%, as overcrowding may lead to reduced viability due to compression.

Related Products

OX40	
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	
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	
Anti-IL-4R hIgG1 Antibody(12B5)	Anti-IL4R hIgG4 Antibody(Dupilumab)
Anti-IL4R hIgG4 Reference Antibody (Dupbio)	
Human IL-4R alpha Protein; mFc Tag	
IL-31	
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
Anti-IL31 hIgG1 Antibody(mAb33)	Anti-IL31RA hIgG1 Antibody(NA633)
Anti-IL31RA hIgG2 Antibody(Nemolizumab)	Anti-OSMR hIgG4 Antibody(Vixarelimab)
MRGPRX2	
H_MRGPRX2 Reporter Cell Line	Cynomolgus_MRGPRX2 CHO-K1 Cell Line
H_MRGPRX2 CHO-K1 Cell Line	H_MRGPRX2 HEK-293 Cell Line

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