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Product Sheet

H_GARP Latent TGFB1 Reporter HEK-293 Cell Line

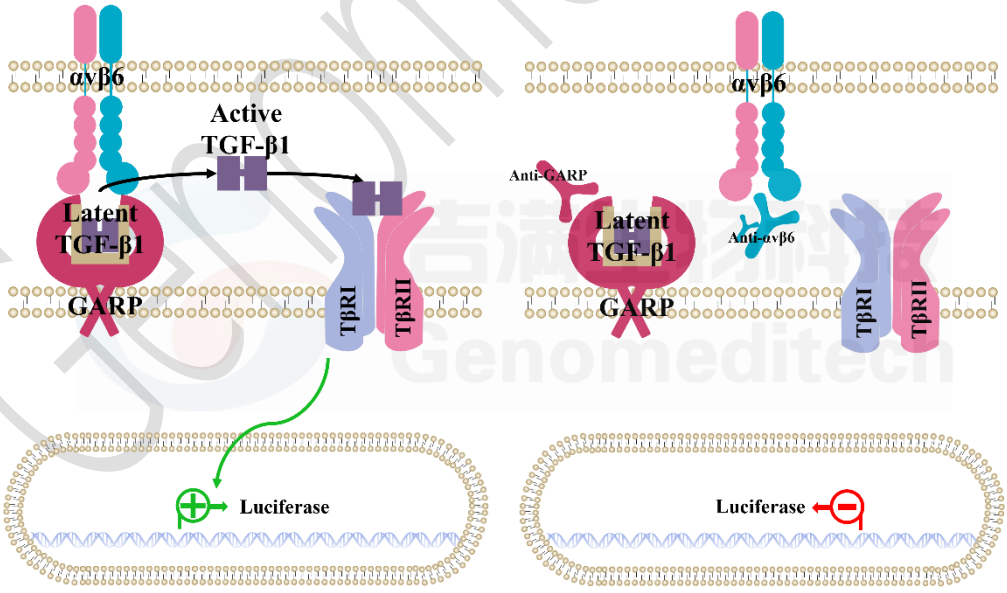
Catalog number: GM-C13361

Version 3.3.1.241206

GARP (Glycoprotein A Repeat-containing Protein) is a type I transmembrane protein mainly found on regulatory T lymphocytes (Tregs) and platelets. It binds to integrin αVβ6/αVβ8, facilitating the release of active TGF-β, which enhances the suppressive function of Tregs and maintains peripheral tolerance.

In the TGF-β signaling pathway, GARP regulates the retention and activation of latent TGF-β complexes. Latent TGF-β1 associates with Tregs, activated B cells, mesenchymal stromal cells, and platelets via LRRC32 (GARP), allowing it to control latent TGF-β retention. GARP's extracellular region binds to latent TGF-β, while integrin αVβ6/αVβ8 recognizes the RGD sequence in LAP, promoting the release of mature TGF-β.

H_GARP Latent TGFB1 Reporter HEK-293 Cell Line is a clonal stable HEK-293 cell line constructed using lentiviral technology, constitutive expression of the GARP, TGFB1 and TβRI gene, along with signal-dependent expression of a luciferase reporter gene. When αVβ6 binds to GARP, promoting the release of Active TGF-β1, 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 GARP.



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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+150 μg/mL Bleomycin+400 μg/mL G418+125 μg/mL Hygromycin+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
Bleomycin	Genomeditech/GM-040407
G418	Genomeditech/GM-040402
Hygromycin	Genomeditech/GM-040403
Puromycin	Genomeditech/GM-040401
H_αvβ6 HEK-293 Cell Line	Genomeditech/GM-C19431
Anti-GARP-TGF-β1 hIgG4 Antibody(ARGX-115)	Genomeditech/GM-30474AB
ONE-Glo™ Luciferase Assay System	Promega/E6120

Figures

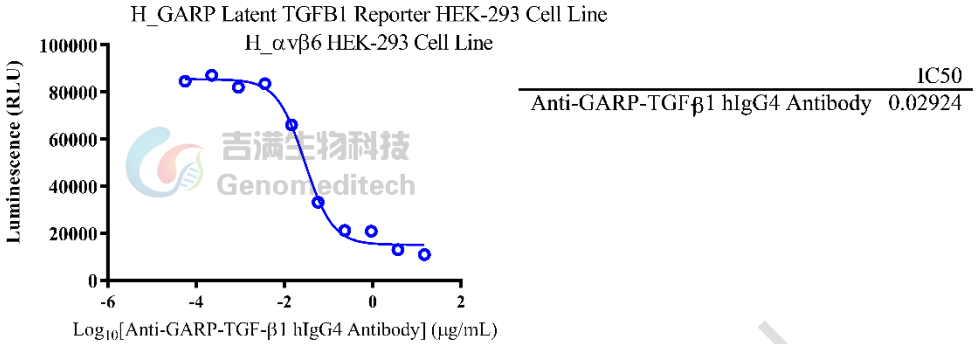


Figure 1 | Response to Anti-GARP-TGF-β1 hIgG4 Antibody(ARGX-115). Serial dilutions of the Anti-GARP-TGF-β1 hIgG4 Antibody(ARGX-115) (Cat. GM-30474AB) were incubated with 2E4 cells/well of the H_GARP Latent TGFB1 Reporter HEK-293 (Cat. GM-C13361) in a 96-well plate for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). Subsequently, the H_αvβ6 HEK-293 (Cat. GM-C19431) at a concentration of 1E4 cells/well was added, and the co-culture proceeded for an additional 6 hours. Firefly luciferase activity is then measured using the ONE-Glo™ Luciferase Assay System (Promega/E6120). The results indicated a maximum blocking fold of approximately [8.4]. Data are shown by drug mass concentration.

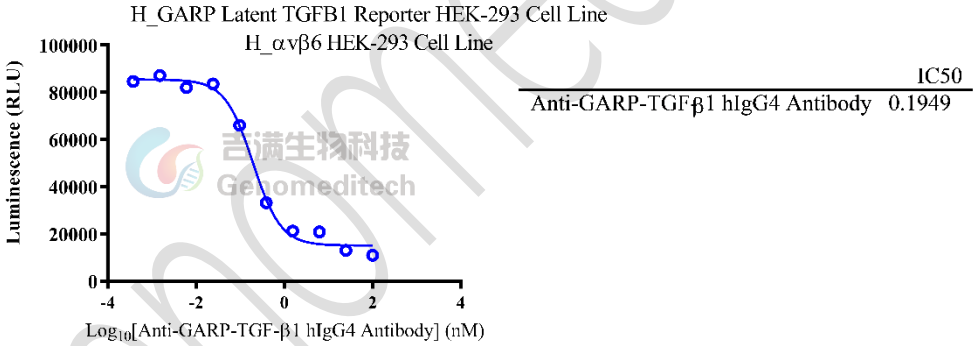
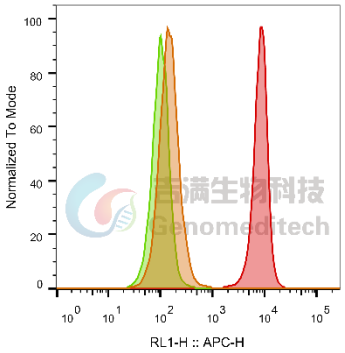


Figure 2 | Response to Anti-GARP-TGF-β1 hIgG4 Antibody(ARGX-115). Serial dilutions of the Anti-GARP-TGF-β1 hIgG4 Antibody(ARGX-115) (Cat. GM-30474AB) were incubated with 2E4 cells/well of the H_GARP Latent TGFB1 Reporter HEK-293 (Cat. GM-C13361) in a 96-well plate for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). Subsequently, the H_αvβ6 HEK-293 (Cat. GM-C19431) at a concentration of 1E4 cells/well was added, and the co-culture proceeded for an additional 6 hours. Firefly luciferase activity is then measured using the ONE-Glo™ Luciferase Assay System (Promega/E6120). The results indicated a maximum blocking fold of approximately [8.4]. Data are shown by drug molar concentration.



	SampleID	Geometric Mean : RL1-H
	HEK-293 anti-GARP-TGF-β1+APC-2nd Ab	147
	H_GARP Latent TGFB1 Reporter HEK-293 H_IgG+APC-2nd Ab	101
	H_GARP Latent TGFB1 Reporter HEK-293 anti-GARP-TGF-β1+APC-2nd Ab	8019

Figure 3 | H_GARP Latent TGFB1 Reporter HEK-293 Cell Line (Cat. GM-C13361) was determined by flow cytometry using Anti-GARP-TGF-β1 hIgG4 Antibody(ARGX-115) (Cat. GM-30474AB).

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.

Cell passage

Growth medium: DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin+150 μg/mL Bleomycin+400 μg/mL G418+125 μg/mL Hygromycin+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

TGF-β:GARP:avβ6	
TGF-β Reporter 293 DDX35TM Cell Line	TGF-β Reporter HEK-293 Cell Line
Cynomolgus_αvβ6 HEK-293 Cell Line	H_GARP HEK-293 Cell Line
H_GARP Latent TGF-β1 CHO-K1 Cell Line	H_GARP Latent TGF-β1 HEK-293 Cell Line
H_ITGB6 CHO-K1 Cell Line	H_ITGB6 HEK-293 Cell Line
H_αvβ6 CT26 Cell Line	H_αvβ6 HEK-293 Cell Line
H_αvβ6 LLC1 Cell Line	H_αvβ6 MC38 Cell Line
Anti-GARP-TGF-β1 hIgG4 Antibody(ARGX-115)	Anti-H_ITGB6 hIgG1 Reference Antibody (h2A2)
Anti-ITGB6 hIgG1 Antibody(SGN-B6A)	Anti-TGFB1 hIgG4 Antibody(SRK-181)
Anti-αv hIgG2 Antibody(Abituzumab)	Anti-αvβ6 hIgG1 Antibody(m15H3)

Anti-ITGB6-MMAE ADC(Dar4)[SGN-B6A]	
ADC Related Product	
Anti-DXD Mouse IgG1 Antibody (23E21C5)	Anti-DXD Mouse IgG1 Antibody (4A5A12)
Anti-Dxd Mouse IgG2a Antibody (17D6A4)	Anti-Eribulin Mouse IgG2a Antibody (10F8G4)
Anti-MMAE Mouse IgG1 Antibody (11C10E3)	Anti-MMAE Mouse IgG2a Antibody (17A1K11)
Anti-MMAE Mouse IgG2a Antibody (8F6A3)	Mouse anti Human IgG-MMAE(Dar4)
Human IgG1 Isotype-DXD (Dar8)	Human IgG1 Isotype-Eribulin (Dar4)
Human IgG1 Isotype-MMAE (Dar4)	
Recombinant DT3C Protein	

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