Genomeditech (Shanghai) Co.,Ltd.
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# Product Sheet

## TGF-β Reporter HEK-293 Cell Line

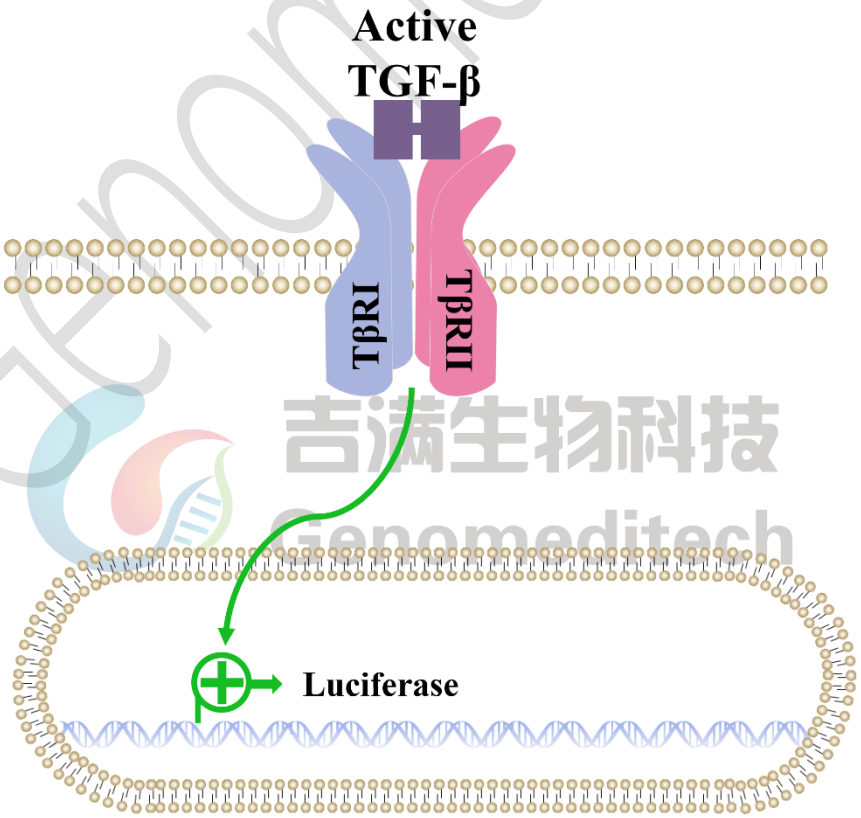
Catalog number: GM-C05346

Version 3.3.1.241030

TGF-Beta (Transforming Growth Factor-Beta) is a type of multifunctional cytokine and a member of the cytokine superfamily. It plays a vital role in various biological processes such as cell growth, differentiation, apoptosis, and immune regulation. The TGF-Beta family includes three homologous isoforms: TGF-β1, TGF-β2, and TGF-β3. While structurally similar, they function differently across various tissues and cell types.

TGF-Beta works by binding to TGF-Beta receptors on cell surfaces, activating downstream signaling pathways. The main pathways include the Smad-dependent and non-Smad pathways. Smad proteins play a critical role in TGF-Beta signal transduction, relocating to the cell nucleus once receptors are activated to regulate the expression of specific genes.

TGF-β Reporter HEK-293 Cell Line is a clonal stable HEK-293 cell line with constitutive expression of the TβRI, endogenously expresses TβRII gene and some adapter membrane molecules, along with signal-dependent expression of a luciferase reporter gene. When TGF-β binds to TGF-β receptors, it activates downstream signaling pathways, leading to the expression of luciferase. 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 this signaling pathway.



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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+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, high glucose, with glutamine | Biological Industries/01-052-1ACS |
| 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 |
| Recombinant Human TGF-beta 1 | Novoprotein/CA59 |
| Luciferase Reporter Gene Aassy Kit(the Kit is replaced by GMOne-Step 2.0) | Genomeditech/GM-040501 |

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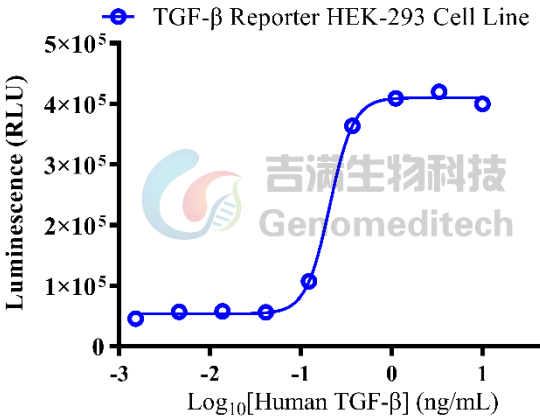
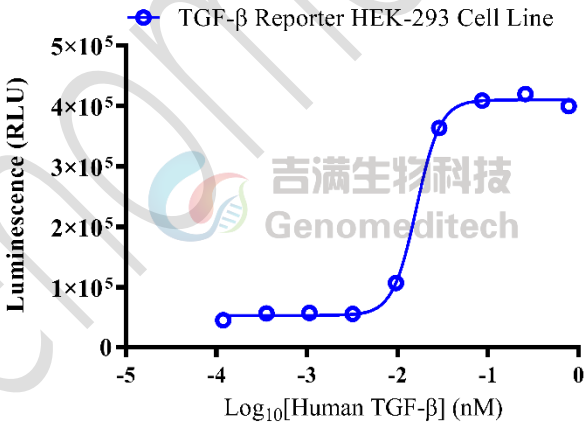
## Figures




| | TGF-β Reporter HEK-293 Cell Line |
|---|---|
| EC50 | 0.2084 |

Figure 1 | Response to Recombinant Human TGF-beta 1. TGF-β Reporter HEK-293 Cell Line (Cat. GM-C05346) at a concentration of 2.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human TGF-beta 1 (novoprotein/CA59) in assay buffer (DMEM +1% FBS +1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Assay System (Cat. GM-040501). The maximum induction fold was approximately [8.0]. Data are shown by drug mass concentration.




| | TGF-β Reporter HEK-293 Cell Line |
|---|---|
| EC50 | 0.01628 |

Figure 2 | Response to Recombinant Human TGF-beta 1. TGF-β Reporter HEK-293 Cell Line (Cat. GM-C05346) at a concentration of 2.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human TGF-beta 1 (novoprotein/CA59) in assay buffer (DMEM +1% FBS +1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Assay System (Cat. GM-040501). The maximum induction fold was approximately [8.0]. Data are shown by drug molar concentration.

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## 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+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 | |
|---|---|
| H_GARP Latent TGFB1 Reporter HEK-293 Cell Line | TGF-β Reporter 293 DDX35TM Cell Line |
| Cynomolgus_αvβ6 HEK-293 Cell Line | H_GARP CHO-K1 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-ITGB6-MMAE ADC(Dar4)[SGN-B6A] |
| 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) |
| 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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