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Product Sheet

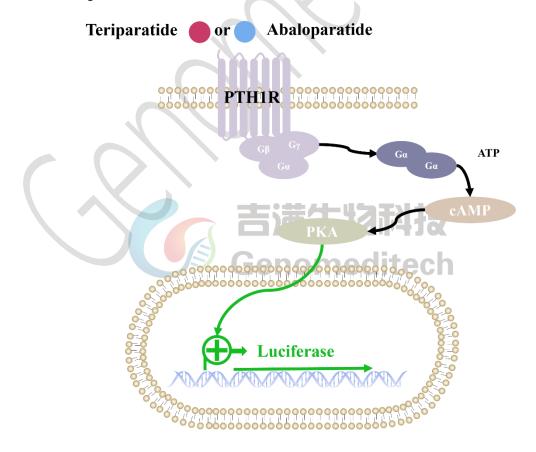
H_PTH1R Reporter 293 Cell Line

Catalog number: GM-C33814

Version 3.3.1.250725

PTH1R (Parathyroid Hormone 1 Receptor) is the primary receptor for parathyroid hormone 1 (PTH) and parathyroid hormone-related peptide (PTHrP), belonging to the G protein-coupled receptor (GPCR) family. In the human body, PTH1R plays a crucial role in the skeleton and kidneys, primarily participating in calcium maintenance, skeletal growth, and metabolic regulation. PTH1R exerts significant effects on bone metabolism by activating the CREB (cAMP Response Element-Binding Protein) signaling pathway. Teriparatide and Abaloparatide are agonists of PTH1R. By binding to PTH1R, they initiate the signaling pathway, enhance osteoblast function, and promote the transcription of genes related to bone metabolism and bone formation. These drugs are used to treat osteoporosis and other bone metabolic disorders.

H_PTH1R Reporter 293 Cell Line is a clonal stable HEK-293 cell line constructed using lentiviral technology, constitutive expression of human PTH1R gene, along with signal-dependent expression of a luciferase reporter gene. The binding of the Teriparatide or Abaloparatide to PTH1R activates downstream reporter genes, leading to luciferase expression. 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 PTH1R.





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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+0.75 μg/mL Puromycin

Note None

Freezing Medium 90% FBS+10% DMSO

Growth properties Adherent

Growth Conditions 37°C, 5% CO₂

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	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
Teriparatide	MCE/HY-P0059
Abaloparatide TFA	MCE/HY-108742A
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

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Figures

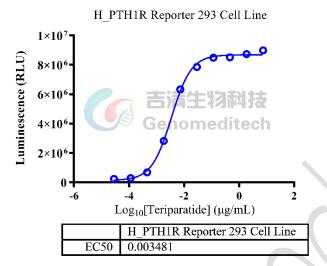


Figure 1 | Response to Teriparatide. The H_PTH1R Reporter 293 Cell Line (Cat. GM-C33814) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Teriparatide (MCE/HY-P0059) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). The maximum induction fold was approximately [33.9]. Data are shown by drug mass concentration.

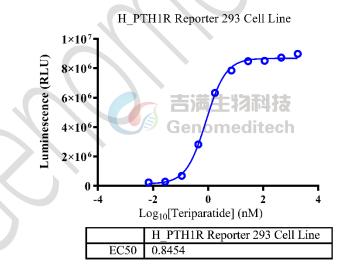


Figure 2 | Response to Teriparatide. The H_PTH1R Reporter 293 Cell Line (Cat. GM-C33814) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Teriparatide (MCE/HY-P0059) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). The maximum induction fold was approximately [33.9]. Data are shown by drug molar concentration.



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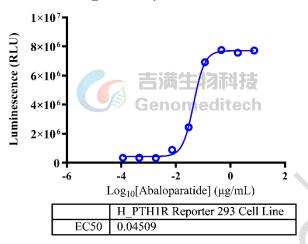


Figure 3 | Response to Abaloparatide. The H_PTH1R Reporter 293 Cell Line (Cat. GM-C33814) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Abaloparatide TFA (MCE/HY-108742A) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). The maximum induction fold was approximately [26.2]. Data are shown by drug mass concentration.

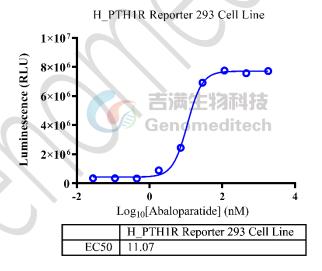


Figure 4 | Response to Abaloparatide. The H_PTH1R Reporter 293 Cell Line (Cat. GM-C33814) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Abaloparatide TFA (MCE/HY-108742A) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). The maximum induction fold was approximately [26.2]. 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% CO₂ 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+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.



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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.

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