

# Product Sheet

## RANK Reporter HEK-293 Cell Line

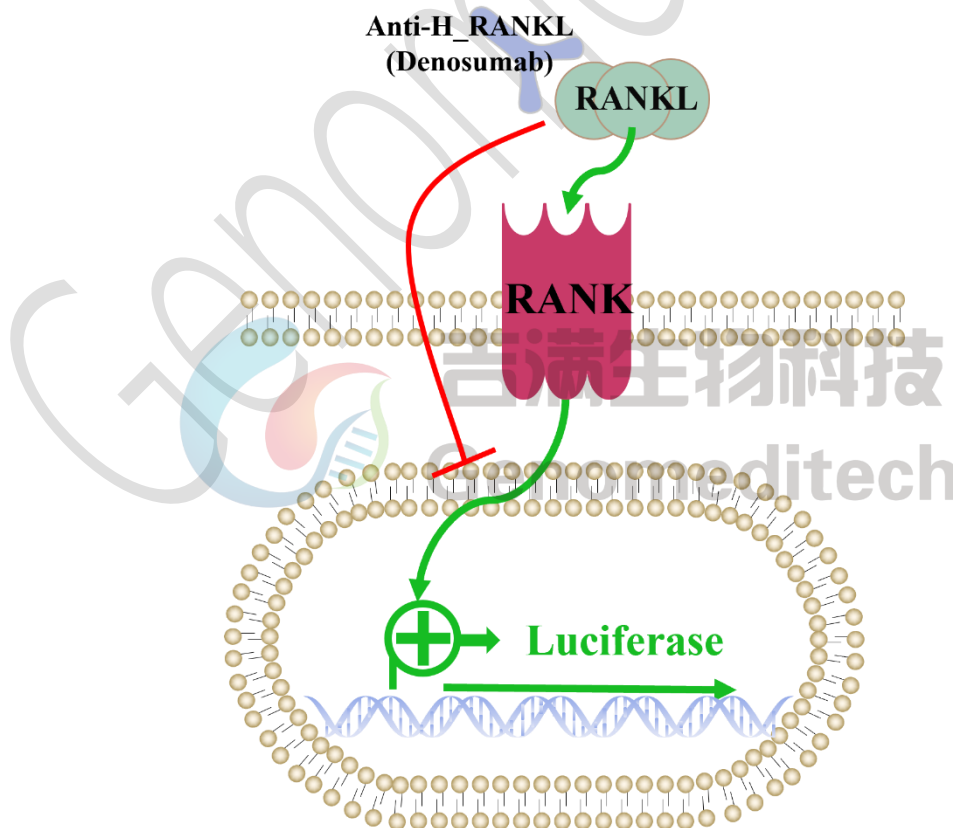
Catalog number: GM-C19909

Version 3.3.1.241218

RANK (Receptor Activator of Nuclear Factor  $\kappa$ B) is a member of the TNF receptor superfamily, crucial for bone metabolism and immune regulation. It is mainly expressed on osteoclast precursors, dendritic cells, and some immune cells. RANK is activated by its ligand RANKL, produced by osteoblasts and activated T cells.

The RANK signaling pathway involves the recruitment of adaptor protein TRAF6 upon RANKL binding, activating downstream cascades such as NF- $\kappa$ B, MAPK, and JNK pathways. Dysregulation of the RANK-RANKL pathway is linked to bone diseases like osteoporosis and rheumatoid arthritis, making it a key target for therapies aimed at modulating bone metabolism and immune responses.

RANK Reporter HEK-293 Cell Line is a clonal stable HEK-293 cell line constructed using lentiviral technology, constitutive expression of the RANK gene, along with signal-dependent expression of a luciferase reporter gene. When RANKL binds to RANK, 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 RANK.



---

## Specifications

---

<b>Quantity</b>	5E6 Cells per vial,1 mL
<b>Product Format</b>	1 vial of frozen cells
<b>Shipping</b>	Shipped on dry ice
<b>Storage Conditions</b>	Liquid nitrogen immediately upon receipt

---

<b>Recovery Medium</b>	DMEM+10% FBS+1% P.S
<b>Growth medium</b>	DMEM+10% FBS+1% P.S+4 µg/mL Blasticidin+0.75 µg/mL Puromycin
<b>Note</b>	None
<b>Freezing Medium</b>	90% FBS+10% DMSO
<b>Growth properties</b>	Adherent
<b>Growth Conditions</b>	37°C, 5% CO <sub>2</sub>

---

<b>Mycoplasma Testing</b>	The cell line has been screened to confirm the absence of Mycoplasma species.
<b>Safety considerations</b>	Biosafety Level 2
<b>Note</b>	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

---

## Materials

---

<b>Reagent</b>	<b>Manufacturer/Catalogue No.</b>
DMEM	Gibco/C11995500BT
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/ <a href="#">GM-040404</a>
Puromycin	Genomeditech/ <a href="#">GM-040401</a>
Recombinant Human RANKL/TNFSF11 Protein	Sino Biological/11682-HNCH
Anti-H_RANKL hIgG2 Antibody(Denosumab)	Genomeditech/ <a href="#">GM-77688AB</a>
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/ <a href="#">GM-040503</a>

---

## Figures

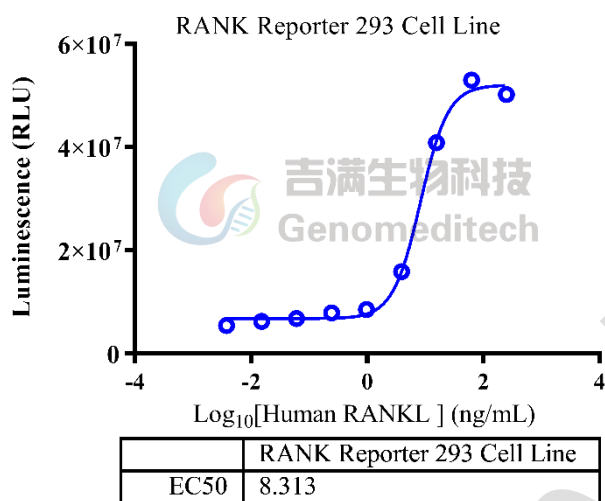


Figure 1 | Response to Recombinant Human RANKL/TNFSF11 Protein. The RANK Reporter HEK-293 Cell Line (Cat. GM-C19909) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human RANKL/TNFSF11 Protein (Sino Biological/11682-HNCH) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 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 [9.3]. Data are shown by drug mass concentration.

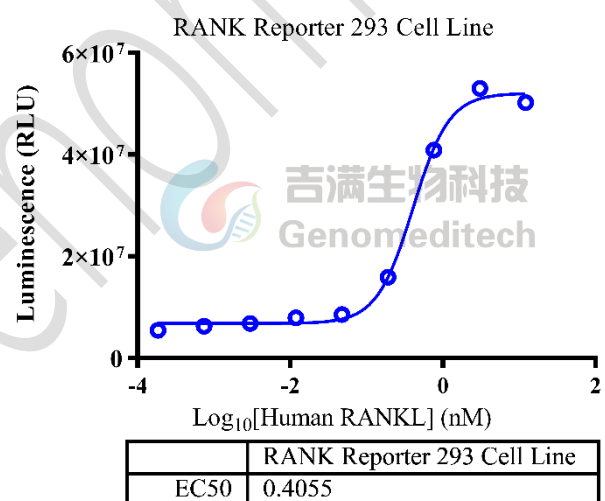


Figure 2 | Response to Recombinant Human RANKL/TNFSF11 Protein. The RANK Reporter HEK-293 Cell Line (Cat. GM-C19909) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human RANKL/TNFSF11 Protein (Sino Biological/11682-HNCH) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 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 [9.3]. Data are shown by drug molar concentration.

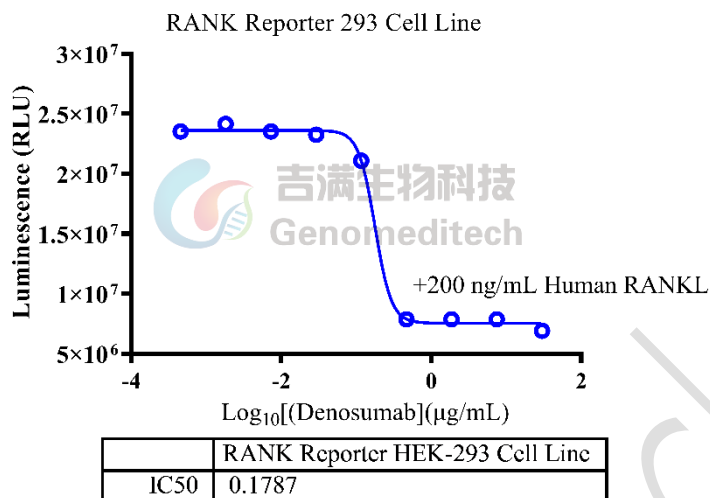


Figure 3 | Response to Anti-H\_RANKL hIgG2 Antibody(Denosumab). Serial dilutions of Anti-H\_RANKL hIgG2 Antibody(Denosumab) (Cat. [GM-77688AB](#)) was incubated with 20 ng/well of Recombinant Human RANKL/TNFSF11 Protein (Sino Biological/11682-HNCH) for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the RANK Reporter HEK-293 Cell Line (Cat. GM-C19909) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 15 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. [GM-040503](#)). The results indicated maximum blocking folds of approximately [3.7]. Data are shown by drug mass concentration.

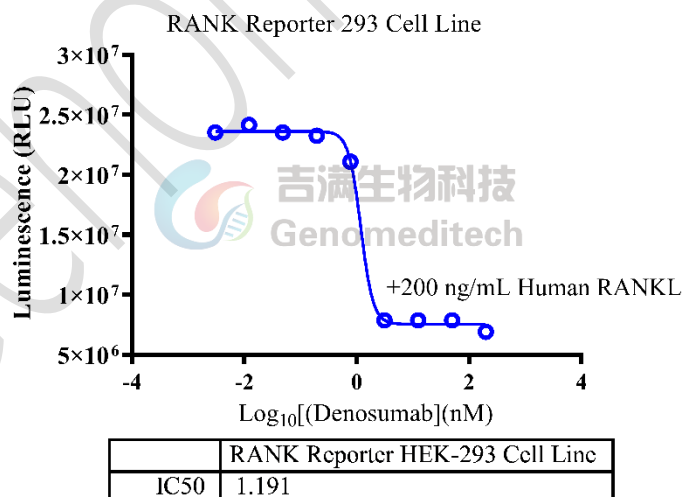


Figure 4 | Response to Anti-H\_RANKL hIgG2 Antibody(Denosumab). Serial dilutions of Anti-H\_RANKL hIgG2 Antibody(Denosumab) (Cat. [GM-77688AB](#)) was incubated with 20 ng/well of Recombinant Human RANKL/TNFSF11 Protein (Sino Biological/11682-HNCH) for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the RANK Reporter HEK-293 Cell Line (Cat. GM-C19909) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 15 hours. Firefly luciferase activity is then measured using

the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. [GM-040503](#)). The results indicated maximum blocking folds of approximately [3.7]. Data are shown by drug molar concentration.

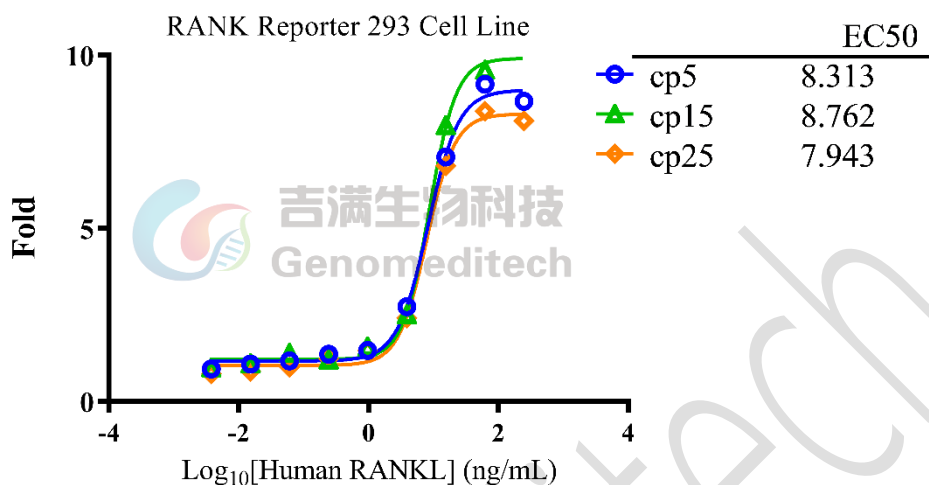


Figure 5 | The passage stability of response to Recombinant Human RANKL/TNFSF11 Protein. The passage 5, 15 and 25 of RANK Reporter HEK-293 Cell Line (Cat. GM-C19909) at a concentration of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Recombinant Human RANKL/TNFSF11 Protein (Sino Biological/11682-HNCH) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 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.

## 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.

- 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).
- 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.
- 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.
- Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.
- Incubate the culture at 37°C in a suitable incubator. A 5% CO<sub>2</sub> in air atmosphere is recommended if using the medium described on this product sheet.

## Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

- Centrifuge at 176 x g for 3 minutes to collect cells.
- Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.
- Aliquot 1 mL into each vial.
- 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.

- 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.
- Remove and discard culture medium.
- Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
- 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).
- 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.
- Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
- After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
- 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

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

## Related Products

RANK RANKL	
<a href="#">Anti-H_RANKL hIgG2 Antibody(Denosumab)</a>	

## Limited Use License Agreement

Genomeditech (Shanghai) Co., Ltd grants to the Licensee all intellectual property rights, exclusive, non-transferable, and non-sublicensable rights of the Licensed Materials; Genomeditech (Shanghai) Co., Ltd will retain ownership of the Licensed Materials, cell line history packages, progeny, and the Licensed Materials including modified materials.

Between Genomeditech (Shanghai) Co., Ltd, and Licensee, Licensee is not permitted to modify cell lines in any way. The Licensee shall not share, distribute, sell, sublicense, or otherwise provide the Licensed Materials, or progenitors to third parties such as laboratories, departments, research institutions, hospitals, universities, or biotechnology companies for use other than for the purpose of outsourcing the Licensee's research.

Please refer to the Genomeditech Cell Line License Agreement for details.

Genomeditech