

Product Sheet

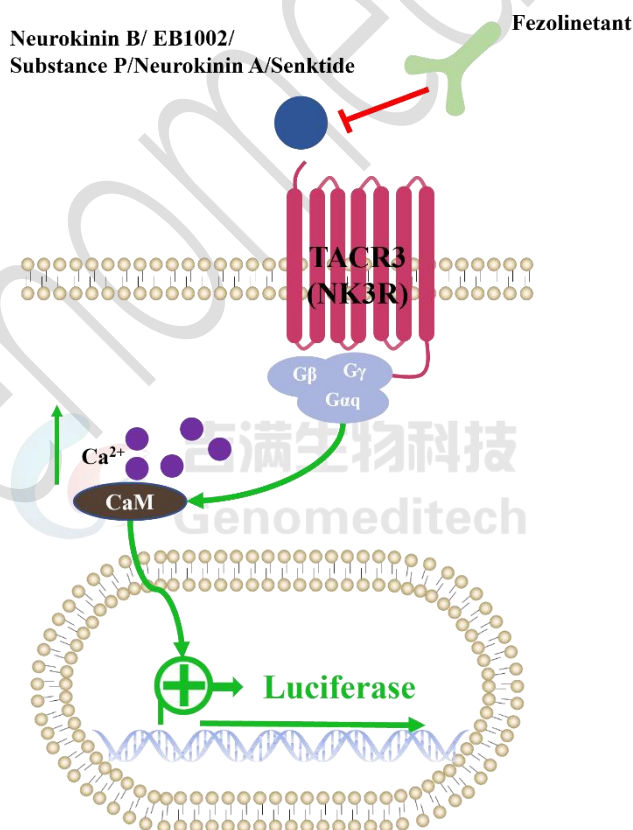
H_TACR3(NK3R) Reporter 293 Cell Line

Catalog number: GM-C39224

Version 3.3.1.26.917

TACR3 (Tachykinin Receptor 3), also known as Neurokinin 3 Receptor (NK3R), belongs to the G protein-coupled receptor (GPCR) family and is widely distributed in both the central nervous system and peripheral tissues. Activation of TACR3 can influence various physiological processes such as neural regulation, endocrine modulation, and reproductive functions. Studies have shown that TACR3 is associated with hypothalamic-pituitary-gonadal axis regulation, pain transmission, the cardiovascular system, and a range of neuropsychiatric disorders. Due to its important physiological roles and disease relevance, TACR3 has emerged as a novel therapeutic target for certain endocrine disorders and neurological diseases.

H_TACR3(NK3R) Reporter 293 Cell Line is a clonal stable 293 cell line constructed using lentiviral technology, constitutive expression of the TACR3(NK3R) gene, along with signal-dependent expression of a luciferase reporter gene. When Neurokinin A or Substance P binds to TACR3(NK3R), 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 TACR3(NK3R).



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% 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	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/ GM-040404
Puromycin	Genomeditech/ GM-040401
Neurokinin A	MCE/HY-P0197
Substance P	MCE/HY-P0201
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/ GM-040513
EB1002	MCE/HY-P10746
Neurokinin B	Aladdin/N118980
Senktide	MCE/HY-P0187
Fezolinetant	MCE/HY-19632

Figures

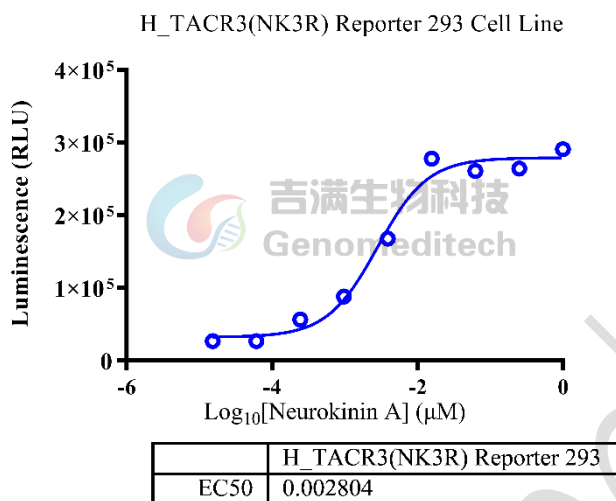


Figure 1 | Response to Neurokinin A. The H_TACR3(NK3R) Reporter 293 Cell Line (Cat. GM-C39224) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Neurokinin A (MCE/HY-P0197) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [11.4]. Data are shown by drug molar concentration.

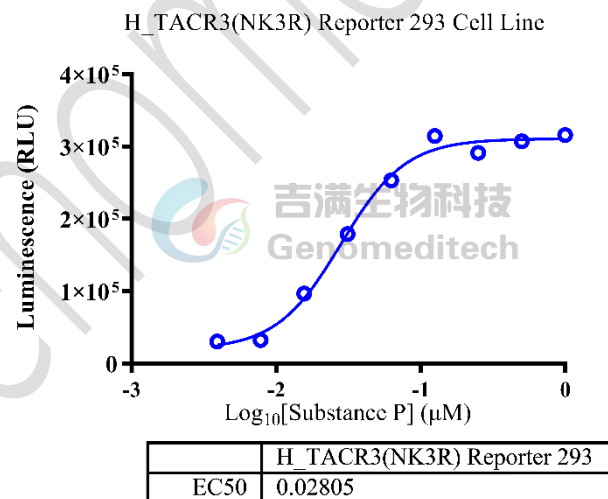


Figure 2 | Response to Substance P. The H_TACR3(NK3R) Reporter 293 Cell Line (Cat. GM-C39224) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Substance P (MCE/HY-P0201) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [10.3]. Data are shown by drug molar concentration.

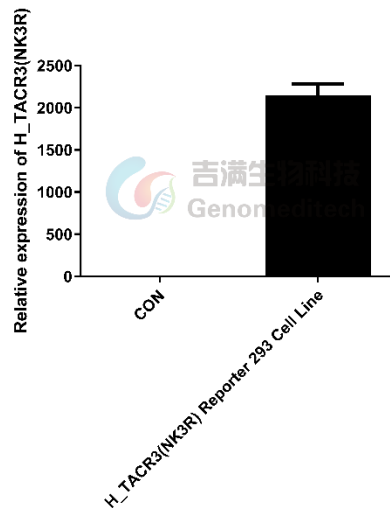


Figure 3 | The mRNA expression levels of H_TACR3(NK3R) Reporter 293 Cell Line (Cat. GM-C39224) were determined by RT-qPCR.

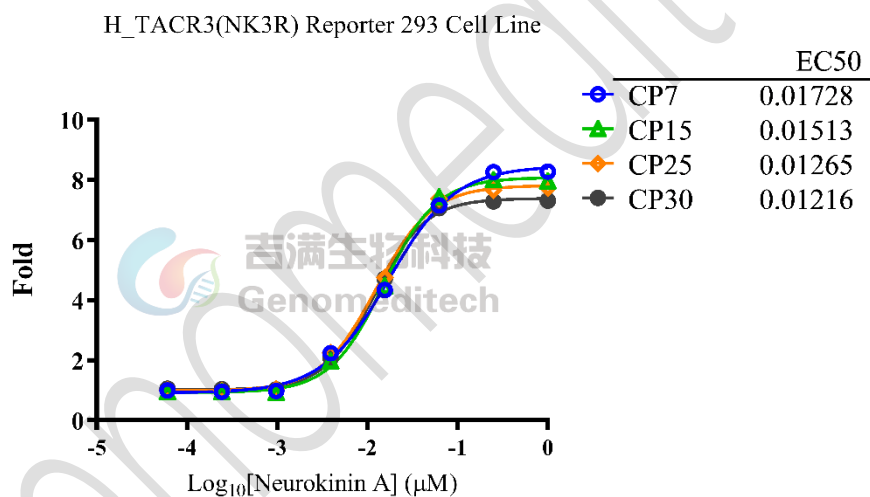


Figure 4 | The passage stability of response to Neurokinin A. The passage 7, 15, 25, and 30 of H_TACR3(NK3R) Reporter 293 Cell Line (Cat. GM-C39224) at a concentration of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Neurokinin A (TargetMol/TP1008) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug molar concentration.

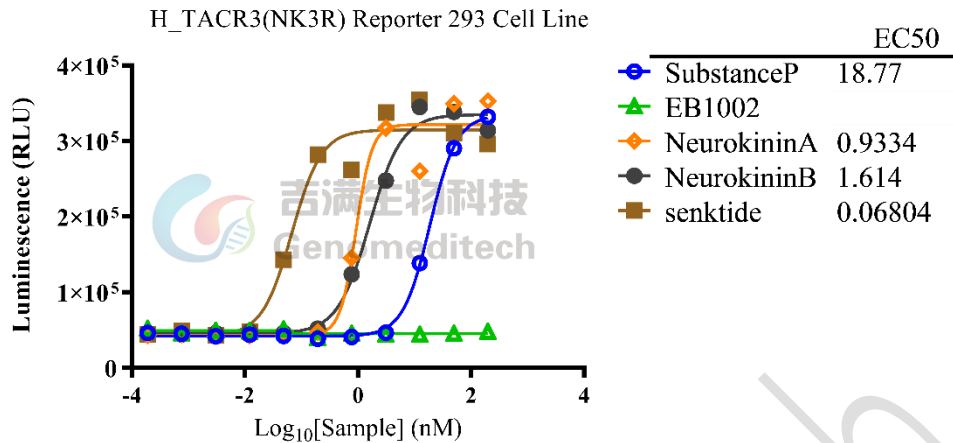


Figure 5 | Response to Substance P, EB1002, Neurokinin A, Neurokinin B, Senktide. The H_TACR3(NK3R) Reporter 293 Cell Line (Cat. GM-C39224) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of NeurokininA(MCE/HY-P0197), SubstanceP(MCE/HY-P0201), EB1002(MCE/HY-P10746), Neurokinin B(aladdin/N118980) and Senktide(MCE/HY-P0187) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [7.6],[7.2],[1.0],[6.8],[6.4]. Data are shown by drug molar concentration.

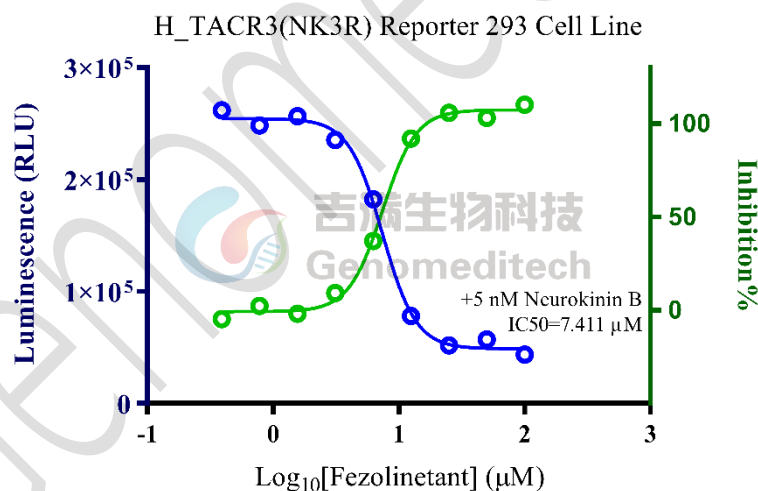


Figure 6 | Inhibition of Neurokinin B-induced reporter activity by Fezolinetant. Serial dilutions of the Fezolinetant (MCE/HY-19632) was incubated with 1.5E4 cells/well of the H_TACR3(NK3R) Reporter 293 Cell Line (Cat. GM-C39224) in a 96-well plate for 1 hour in assay buffer (DMEM +1% FBS+1% P.S). Subsequently, the Neurokinin B(aladdin/N118980) was added to each well to a final concentration of 5 nM, and the coculture proceeded for an additional 6 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit (Genomeditech)(left Y-axis, relative luminescence units), with inhibition percentages shown on the right Y-axis.

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% CO₂ 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.

- 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

TACR2(NK2R)	
H_TACR2(NK2R) Gq Reporter 293 Cell Line	H_TACR2(NK2R) Gs Reporter 293 Cell Line
TACR1(NK1R)	
H_TACR1(NK1R) Reporter 293 Cell Line	

License Agreement:

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