Genomeditech (Shanghai) Co.,Ltd.
Order: +86 021-68455258/50432826/50432825
Toll-free: +86 400 627 9288
Email: service@genomeditech.com

Product Sheet

H_TrkB Reporter CHO-K1 Cell Line

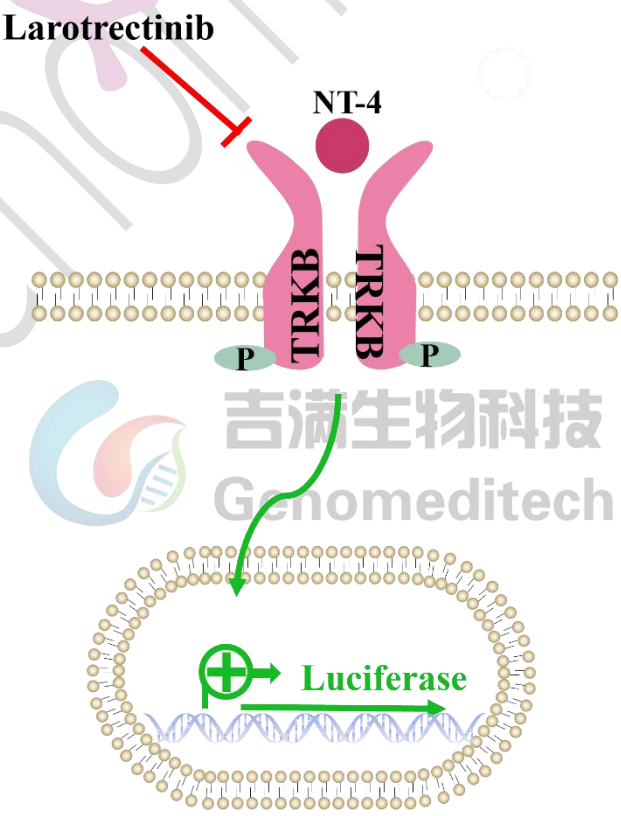
Catalog number: GM-C28366

Version 3.3.1.260610

Tropomyosin receptor kinase B (TrkB), encoded by the NTRK2 gene, is a receptor tyrosine kinase predominantly expressed on neurons in the central and peripheral nervous systems. It serves as the high-affinity receptor for brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5), playing a pivotal role in neuronal survival, synaptic plasticity, and neurite outgrowth, making it a promising therapeutic target for neurodegenerative diseases and depression.

Upon NT-4 binding, TrkB undergoes dimerization and autophosphorylation, initiating multiple downstream signaling cascades including the PLCγ, Ras/MAPK, and PI3K/AKT pathways. PLCγ activation leads to PIP_2 hydrolysis, generating IP_3 and DAG, which elevate intracellular calcium and activate PKC, ultimately converging on NF-κb and CREB to drive pro-survival gene transcription and synaptic remodeling.

H_TrkB Reporter CHO-K1 Cell Line is a clonal stable CHO-K1 cell line constructed using lentiviral technology, constitutive expression of the TrkB gene, along with signal-dependent expression of a luciferase reporter gene. When NT-4 or other TrkB activators bind to TrkB, 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 TrkB.



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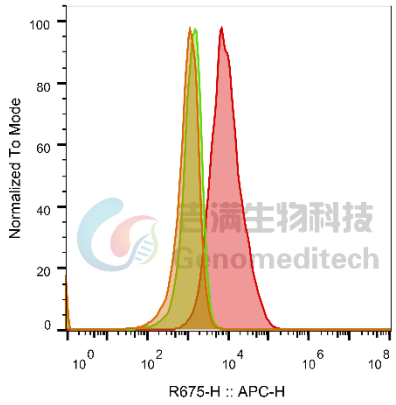
Specifications

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	F12K+10% FBS+1% P.S
Growth medium	F12K+10% FBS+1% P.S+4 μg/mL Blasticidin+4 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

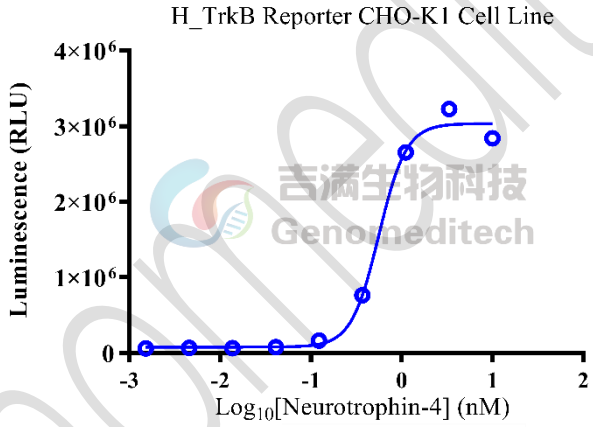
Reagent	Manufacturer/Catalogue No.
F12K	BOSTER/PYG0036
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
Anti-TrkB hIgG4 Antibody (H4H9816P2)	Genomeditech/GM-88117AB
Neurotrophin-4, Human (HEK293)	MCE/HY-P702537
Larotrectinib	MCE/HY-12866
Selitrectinib	MCE/HY-101977
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

Figures



SampleID	Geometric Mean : R675-H
CHO-K1 Anti-Trkb+APC 2nd Ab	870
H_TrkB Reporter CHO-K1 H_IgG+APC 2nd Ab	1141
H_TrkB Reporter CHO-K1 Anti-Trkb+APC 2nd Ab	9048

Figure 1 | H_TrkB Reporter CHO-K1 Cell Line (Cat. GM-C28366) was determined by flow cytometry using Anti-TrkB hIgG4 Antibody (H4H9816P2))(Cat. GM-88117AB).



	H_TrkB Reporter Cell Line
EC50	0.5615

Figure 2 | Response to Neurotrophin-4 protein. H_TrkB Reporter CHO-K1 Cell Line (Cat. GM-C28366) at a concentration of 1E4 cells/well (96-well format) was stimulated with serial dilutions of Neurotrophin-4 Protein (MCE/ HY-P702537) in assay buffer (F12K + 1% FBS + 1% P.S) for 7 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [50.0]. Data are shown by drug mass concentration.

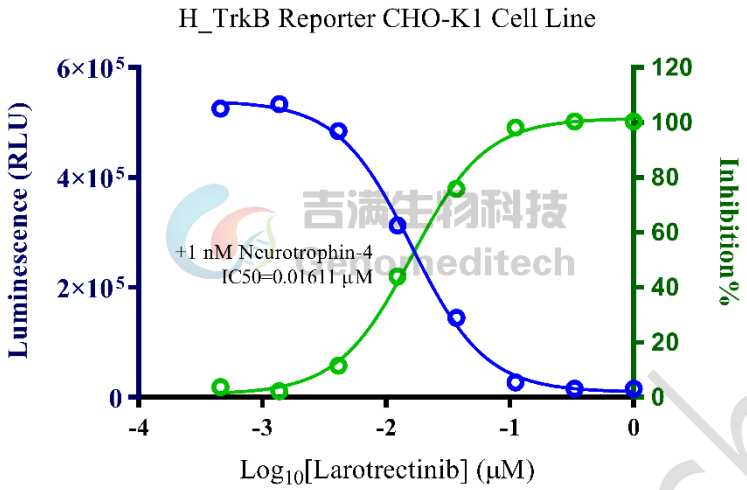


Figure 3 | Inhibition of Neurotrophin-4, Human (HEK293) protein-induced reporter activity by Larotrectinib. Serial dilutions of the Larotrectinib(MCE/HY-12866) was incubated with 1E4 cells/well of the H_TrkB Reporter CHO-K1 Cell Line (Cat. GM-C28366) in a 96-well plate for 1 hour in assay buffer (F12K +1% FBS+1% P.S). Subsequently, the Neurotrophin-4, Human (HEK293) Protein (MCE/HY-P702537) at a concentration of 1 nM/well was added, 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.

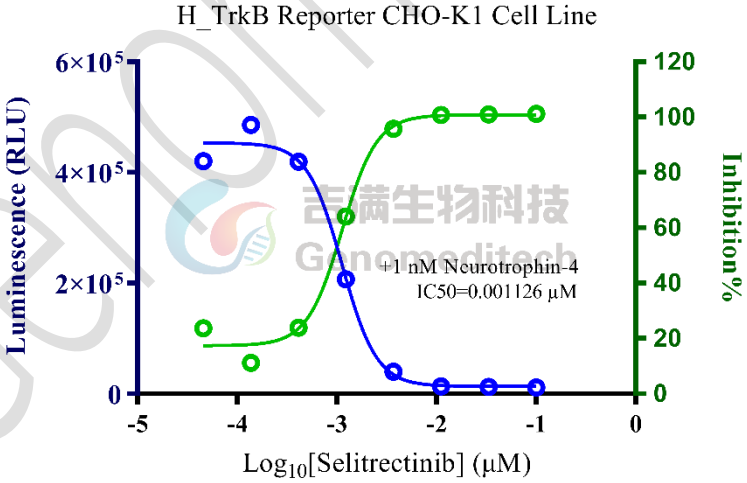


Figure 4 | Inhibition of Neurotrophin-4, Human (HEK293) protein-induced reporter activity by Selitrectinib. Serial dilutions of the Selitrectinib(MCE/HY-101977) was incubated with 1E4 cells/well of the H_TrkB Reporter CHO-K1 Cell Line (Cat. GM-C28366) in a 96-well plate for 1 hour in assay buffer (F12K +1% FBS+1% P.S). Subsequently, the Neurotrophin-4, Human (HEK293) Protein (MCE/HY-P702537) at a concentration of 1 nM/well was added, 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: F12K+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: F12K+10% FBS+1% P.S+4 μg/mL Blasticidin+4 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) Remove and discard culture medium.
b) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
c) 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 2 to 3 minutes at 37°C).
d) 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.

e) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.

f) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.

g) Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 - 1:5 is recommended

Medium Renewal: Every 2 to 3 days

Notes

a) After the stabilization of the cell condition, there will be fewer dead cells post-passage, the cell growth rate will tend to stabilize, cell morphology will become uniform, and the cells will appear robust.

Related Products

TRKB	
Anti-TrkB hIgG4 Antibody (H4H9816P2)	

License Agreement:

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