Description

TrkA/ SRE Luciferase Reporter HEK293 Cell Line is a HEK293 cell line that contains a firefly luciferase reporter under the control of Serum Response Elements (SRE) and constitutive expression of human TrkA (tropomyosin receptor kinase A, NM_002529.3).

This cell line has been validated by flow cytometry and has been shown to be responsive to NGF (nerve growth factor) and inhibited by Larotrectinib (also known as LOXO101 or Vitrakvi™) and an anti-NGF antibody.

Background

A chronic pain mediator, NGF (Nerve Growth Factor) binds to TrkA (tropomyosin receptor kinase A) resulting in activation of the downstream signaling pathway linked to pro-nociception. TRKs are a family of tyrosine receptor kinases, which include TrkA, TrkB and TrkC. TrkA is activated by binding to nerve growth factor (NGF), resulting in activation of cell proliferation through the RAS (Rat sarcoma virus)/MAPK (mitogen-activated protein kinase)/ERK (extracellular signal-regulated kinases) and PLCγ (phospholipase C gamma)/PI3K (phosphoinositide 3-kinase) pathways. Constitutive activation of TrkA is associated with colorectal cancer. Targeting the kinase activity of TrkA and the interaction between NGF/TrkA have been attractive tools in pain management research and cancer.

Application

- Monitor NGF -induced activity.
- Screen molecules that block the NGF/TrkA signaling pathway.
- Screen inhibitors of TrkA kinase activity.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ≥1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1A	BPS Bioscience #79528

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium: Assay Medium 1A	BPS Bioscience 79805
Recombinant Human beta-NGF Protein	R&D System #256-GF-100
Larotrectinib (LOXO101)	
Anti-Beta NGF (Nerve Growth Factor) Neutralizing Antibody	BPS Bioscience #100436
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate and 1% Penicillin/Streptomycin.

Growth Medium 1A (BPS Bioscience #79528):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 100 µg/ml Hygromycin B and 400 µg/ml Geneticin®, G418 Sulfate.

Media Required for Functional Cellular Assay

Assay Medium 1A (BPS Bioscience #79805):
Opti-MEM plus 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 1 to the conical tube containing the cells. Thaw Medium 1 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. Cells should be passaged when they are 85-90% confluent. At first passage and subsequent passages, use Growth Medium 1A.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1A and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1A.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5-1:10 once or twice a week.

   Note: Just after thawing, the cells may grow at a slower rate. It is recommended to split the cells with ~ 1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with higher ratio.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+} and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1A and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at >1 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

Validation Data

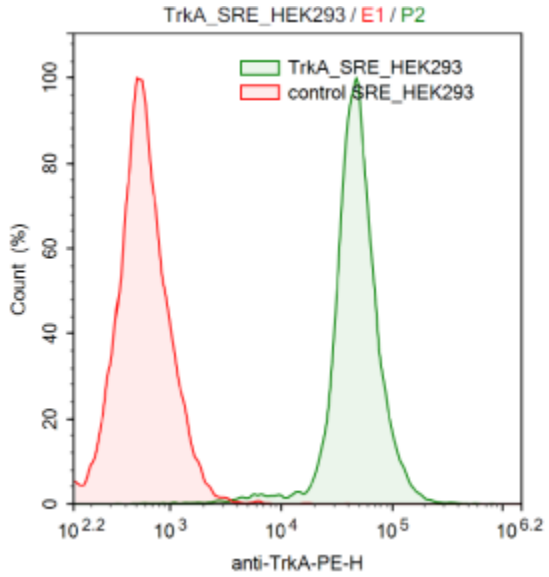

Figure 1: Expression assessment of TrkA in TrkA/ SRE Luciferase Reporter HEK293 Cell Line by flow cytometry.
TrkA/ SRE Luciferase Reporter HEK293 cells (green) or control SRE-HEK293 cells (red) were stained with Human TrkA PE-conjugated Antibody (R&D Systems #FAB1751P) and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

Functional Validation

- The following assays were designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.

- Assay A should include "Stimulated Cells" (or "Test Compound"), "Background Control" and "Unstimulated Control" conditions.
- Assay B should include "Test Inhibitor", "Background Control" and "Untreated Control" conditions.
- If using inhibitors soluble in DMSO do not exceed a final concentration of DMSO of 0.1%.
- Assay C should include "No NGF, No Antibody", "NGF, No Antibody", "NFG, Antibody" and "Background Control" conditions.

A. Dose Response of TrkA/ SRE Luciferase Reporter HEK293 Cell Line to human NGF

1. Seed cells at a density of 50,000 cells per well in 90 µl of Assay Medium 1A into a white clear-bottom 96-well microplate. Leave a few empty wells as cell-free control wells ("Background Control").
2. Incubate the cells in a 5% CO_2 incubator at 37°C overnight (~16 hours).
3. Next day, prepare a two-fold serial dilution of human NGF in Assay Medium 1A at 10x the final testing concentrations (10 µl/well).
4. Add 10 µl of diluted NGF to the "Stimulated Cells" wells.
5. Add 10 µl of Assay Medium 1A to the "Unstimulated Control" wells.
6. Add 100 µl of Assay Medium 1A to "Background Control" wells (cell-free wells).
7. Incubate at 37°C with 5% CO_2 for ~ 6 hours.
8. Add 100 µl of ONE-Step™ Luciferase reagent per well.
9. Incubate at Room Temperature (RT) for ~15-30 minutes.
10. Measure luminescence using a luminometer.
11. The "Background Control" luminescence value should be subtracted from all readings.
12. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

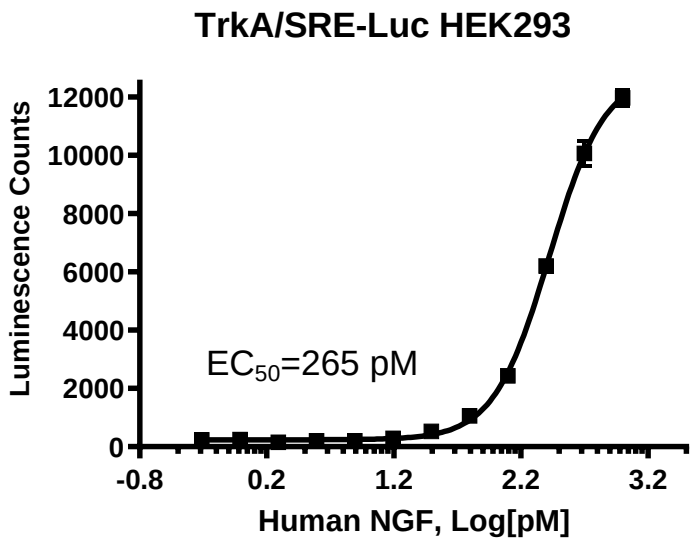

Figure 2. Dose response curve of TrkA/ SRE Luciferase Reporter HEK293 Cell Line to NGF.
TrkA/ SRE Luciferase Reporter HEK293 cells were treated with increasing concentrations of NGF. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to cells without treatment (unstimulated control).

B. Inhibition of TrkA kinase activity in TrkA/ SRE Luciferase Reporter HEK293 Cell Line

1. Seed cells at a density of 50,000 cells per well in 80 µl of Assay Medium 1A into a white clear-bottom 96-well microplate. Leave a few empty wells as cell-free control wells ("Background Control").
2. Incubate the cells in a 5% CO_2 incubator at 37°C overnight (~16 hours).
3. Prepare a serial dilution of Larotrectinib (LOXO101) in Assay Medium 1A at 10x the final concentration (10 µl/ well).
4. Add 10 µl of diluted LOXO101 to the "Test Inhibitor" wells.
5. Add 10 µl of Assay Medium 1A to the "Untreated Control" wells.
6. Add 100 µl of Assay Medium 1A to "Background Control" wells (cell-free wells).
7. Incubate at 37°C with 5% CO_2 for ~ 1 hours.
8. Prepare a solution of NGF at 10x the calculated EC_{90} value (10 µl/ well).
9. Add 10 µl of NGF to each well.
10. Incubate at 37°C with 5% CO_2 for ~ 5 hours.

11. Add 100 µl of ONE-Step™ Luciferase reagent per well.

12. Incubate at RT for ~15-30 minutes.

13. Measure luminescence using a luminometer.

14. The "Background Control" luminescence value should be subtracted from all readings.

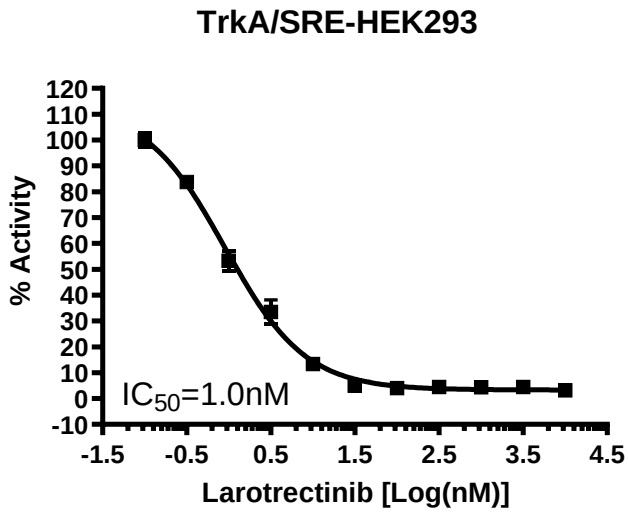

Figure 3. Inhibition of TrkA/ SRE Luciferase Reporter HEK293 Cell Line by Larotrectinib (LOX101). TrkA/ SRE Luciferase Reporter HEK293 cells were treated with increasing concentrations of Larotrectinib (LOX101). Luciferase activity was measured using the ONE-Step™ Luciferase Assay System.

C. Inhibition of NGF/TrkA induced signaling in TrkA/ SRE Luciferase Reporter HEK293 Cell Line

1. Seed cells at a density of 50,000 cells per well in 90 µl of Assay Medium 1A into a white clear-bottom 96-well microplate. Leave a few empty wells as cell-free control wells ("Background Control").

2. Incubate the cells in a 5% CO_2 incubator at 37°C overnight (~16 hours).

3. Prepare Assay Medium 1A containing NGF at 10x the calculated EC_{90} value. This will Assay Medium Mix.

4. Prepare a serial dilution of Anti-NGF Antibody in Assay Medium Mix at 10x the final concentration (10 µl/ well) and incubate for 45 minutes at RT.

5. Add 10 µl of diluted Anti-NGF Antibody to the "NGF, Antibody" wells.

6. Add 10 µl of Assay Medium Mix to the "NGF, No Antibody" wells.

7. Add 10 µl of Assay Medium 1A to the "No NGF, No Antibody" wells.

8. Add 100 µl of Assay Medium Mix to "Background Control" wells (cell-free wells).
9. Incubate at 37°C with 5% CO_2 for ~ 5 hours.
10. Add 100 µl of ONE-Step™ Luciferase reagent per well.
11. Incubate at RT for ~15-30 minutes.
12. Measure luminescence using a luminometer.
13. The "Background Control" luminescence value should be subtracted from all readings.

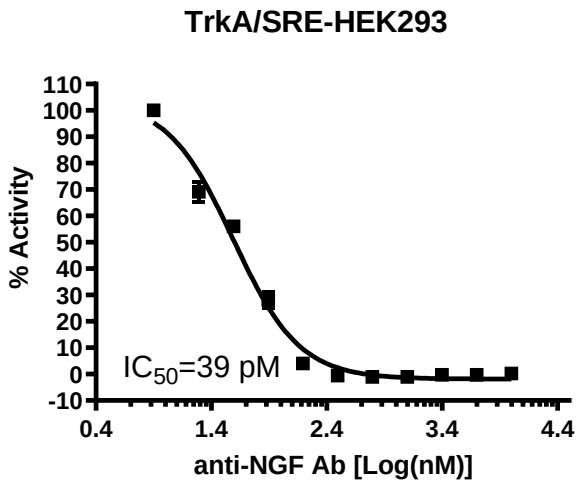

Figure 4. Inhibition of TrkA/ SRE Luciferase Reporter HEK293 Cell Line by Anti-Beta NGF (Nerve Growth Factor) Neutralizing Antibody.
TrkA/ SRE Luciferase Reporter HEK293 cells were treated with increasing concentrations of Anti-Beta NGF (Nerve Growth Factor) Neutralizing Antibody (#100436) in the presence of NGF. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System.

Data shown is representative.

Sequence

Human TrkA sequence (accession number NM_002529.3)

MLRGGRRGQLGWHSWAAGPGSLLAWLILASAGAAPCPDACCPHGSSGLRCTRDGALDSLHHLPGAENLTELYIENQQHLQHLE
LRDLRGLGELRNLTIVKSGLRFVAPDAFHFTPRLSRLNLSFNALESLSWKTVQGLSLQELVLSGNPLHCSCALRWLQRWEEEGLGG
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SRDIYSTDYYRVGGRTMLPIRWMPPESILYRKFTTESDVWSFGVVLWEIFTYGKQPWYQLSNTEAIDCITQGRELERPRACPPEVY
AIMRGCWQREPQQRHSIKDVHARLQALAQAPPVYLDVLG

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Troubleshooting Guide
For lot-specific information and all other questions, please email visit https://bpsbioscience.com/contact. Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions.

Related Products

Products	*Catalog #*	*Size*
TrkA Assay Kit	79548	96 reactions
Human beta Nerve Growth Factor Recombinant	90223	5 µg/ 20µg

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