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Data Sheet

TrkA / SRE Reporter – HEK293 Recombinant Cell Line

Catalog #: 79798

Product Description
Recombinant HEK-293 cells expressing firefly luciferase gene under the control of Serum Response Elements (SRE) with constitutive expression of human TrkA (Tropomyosin receptor kinase A; NTRK1; ref. seq. NM_002529.3).

Background
A chronic pain mediator, NGF (Nerve Growth Factor) binds to TrkA resulting in activation of the downstream signaling pathway linked to pro-nociception; therefore, targeting the kinase activity of TrkA and the interaction between NGF/TrkA have been attractive tools in pain management research. In addition to control of chronic pain, a recent clinical success has proven that inhibition of TrkA kinase would be a promising anti-cancer strategy.

Application
- Screen for inhibitors of NGF/TrkA signaling in a cellular context
- Screen for inhibitors of TrkA kinase activity in a cellular context

Format
Each vial contains 2 x 10^6 cells in 1 ml of 10% DMSO in FBS

Storage
Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing
The cell line has been screened using the MycoAlert™ Mycoplasma Detection kit (Lonza, #LT07-218) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 1 (BPS Bioscience, #60187): MEM medium (Hyclone, #SH30024.01) supplemented with 10% FBS (Invitrogen, #26140-079), 1% non-essential amino acids (Hyclone, #SH30238.01), 1 mM Na pyruvate (Hyclone, #SH30239.01), 1% Penicillin/Streptomycin (Hyclone, SV30010.01)

Growth Medium 1A (BPS Bioscience, #79528): Thaw Medium 1 (BPS Bioscience, #60187) plus 400 µg/ml of Geneticin (Life Technologies #11811031) and 100 µg/ml of Hygromycin B (Life Technologies, #10687-010)

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1A.

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Assay Medium 1A (BPS Bioscience #79805): Opti-MEM (Thermo Fisher, #31985070) plus 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin and Hygromycin B)**. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 **(no Geneticin and Hygromycin B)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. After 24 hours of culture, add an additional ~3 ml of Thaw Medium 1 (**no Geneticin and Hygromycin B)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach ~2.5 x 10^6 cells/ml. At first passage, switch to Growth Medium 1A **(contains Geneticin and Hygromycin B).**

To passage the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Growth Medium 1B (contains Geneticin) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 1B (contains Geneticin) and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ration: 1:5 to 1:10 weekly 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.

To freeze down the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 1 (no Geneticin) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) to ~2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage.

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

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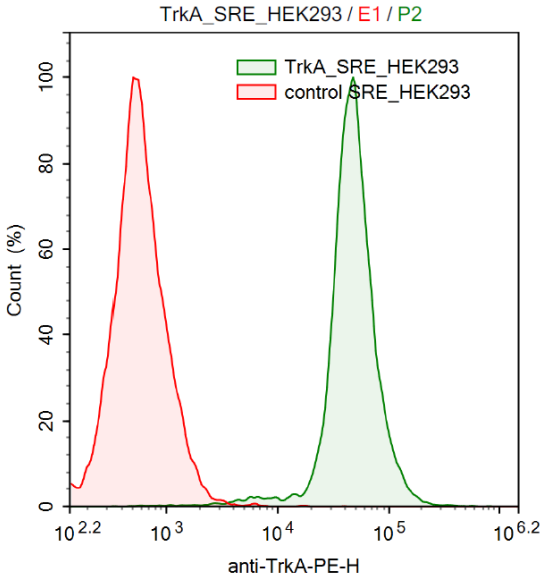
Functional Validation and Assay Performance



TrkA/SRE-HEK293 cells (green) or control SRE-HEK293 cells (red) were stained with PE-labeled Anti-TrkA Antibody (R&D systems, #FAB1751P) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.

The following assays are designed for 96-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.

Materials Required but Not Supplied

- Human NGF (R&D Systems, #256-GF-100)
- Media: Thaw Medium 1 (BPS Bioscience, #60187), Growth Medium 1A (BPS Bioscience, #79528), Assay Medium 1A (BPS Bioscience, #79805)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- ONE-Step™ Luciferase Assay System (BPS Bioscience, #60690)
- Luminometer

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A. Dose response of TrkA/SRE Reporter-HEK293 cells to human NGF

1. Harvest TrkA/SRE Reporter-HEK293 cells from culture in the Growth Medium 1A and seed cells at a density of ~50,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of Assay Medium 1A. Leave a couple of wells empty for use as a cell-free control.

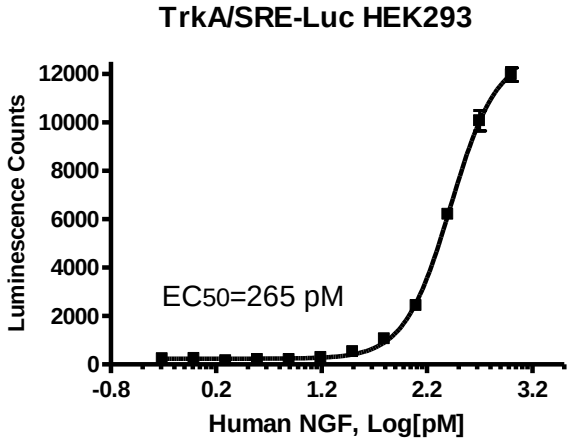
2. Incubate cells at 37°C in a CO_2 incubator for ~ 16 hours.

3. Add 10 µl of two-fold serial dilution of human NGF protein (R&D Systems, #256-GF-100) in the Assay Medium 1A to stimulated wells. Add 10 µl of Assay Medium 1A to the unstimulated control wells. Add 100 µl of Assay Medium 1A to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate. Incubate the plate at 37°C in a CO_2 incubator for 6 hours.

4. Perform luciferase assay using ONE-Step Luciferase Assay buffer, according to the recommended instructions: Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.

5. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

Example: **Dose response of TrkA/SRE-HEK293 cells to human NGF**



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B. Inhibition of kinase activity of TrkA in TrkA/SRE Reporter-HEK293 cells

1. Harvest TrkA/SRE Reporter-HEK293 cells from culture in the Growth Medium 1A and seed cells at a density of ~50,000 cells per well into a white clear-bottom 96-well microplate in 80 µl of Assay Medium 1A. Leave a couple of wells empty for use as a cell-free control.

2. Incubate cells at 37°C in a CO_2 incubator for ~ 16 hours.

3. Add 10 µl TrkA inhibitor or vehicle control in 10 µl of Assay Medium 1A to the wells. (Do not exceed a final DMSO concentration above 0.1%)

4. Incubate cells at 37°C in a CO_2 incubator for ~ 1 hours. Add 10 µl NGF protein at final concentration of EC50 in the Assay Medium 1A to the wells.

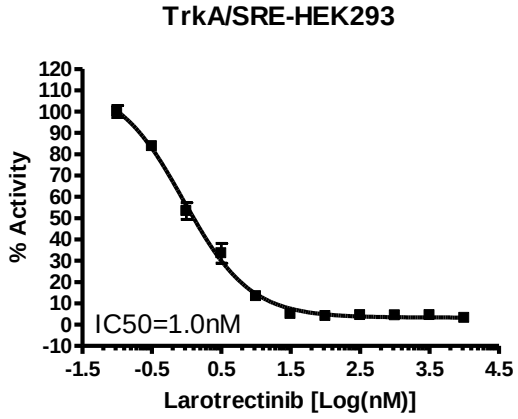
5. Add 100 µl of Assay Medium 1A to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.

6. Incubate the plate at 37°C in a CO_2 incubator for 5 hours.

7. Perform luciferase assay using ONE-Step™ Luciferase Assay kit, according to the recommended instructions: Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.

8. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

Example: **Inhibition of TrkA by Larotrectinib (LOXO101) in TrkA/SRE-HEK293 cells**



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C. Inhibition of NGF/TrkA signaling in TrkA/SRE Reporter-HEK293 cells

1. Harvest TrkA/SRE Reporter-HEK293 cells from culture in the Growth Medium 1A and seed cells at a density of ~50,000 cells per well into a white clear-bottom 96-well microplate in 90 µl of Assay Medium 1A. Leave a couple of wells empty for use as a cell-free control.

2. Incubate cells at 37°C in a CO_2 incubator for ~ 16 hours.

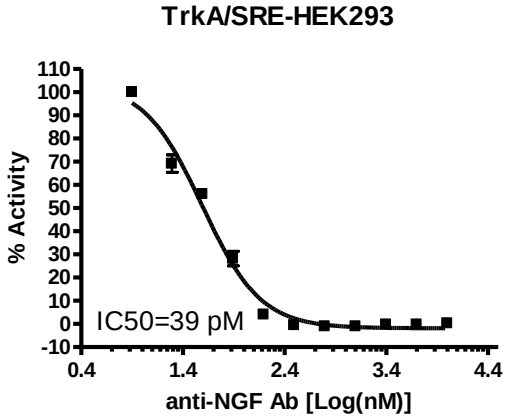
1. Prepare 100 µl serially diluted anti-NGF antibody plus NGF (at 10X EC50 concentration) solution in the Assay Medium 1A and incubate for 45 minutes at room temperature.

2. Add 10 µl the anti-NGF antibody/NGF mixture to the cells and incubate the plate at 37°C in a CO_2 incubator for 5 hours.

3. Perform luciferase assay using ONE-Step™ Luciferase Assay kit, according to the recommended instructions: Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.

4. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

Example: **Inhibition of NGF/TrkA signaling by anti-NGF antibody in TrkA/SRE-HEK293 cells**



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Sequence
Human TrkA sequence (accession number NM_002529.3)

MLRGGRRGQLGWHSWAAGPGSLLAWLILASAGAAPCPDACCPHGSSGLRCTRDGALDSLHH
LPGAENLTELYIENQQHLQHLELRDLRGLGELRNLTIVKSGLRFVAPDAFHFTPRLSRLNLSFNA
LESLSWKTVQGLSLQELVLSGNPLHCSCALRWLQRWEEEGLGGVPEQKLQCHGQGPLAHMP
NASCGVPTLKVQVPNASVDVGDDVLLRCQVEGRGLEQAGWILTELEQSATVMKSGGLPSLGL
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LAVASQVAAGMVYLAGLHFVHRDLATRNCLVGQGLVVKIGDFGMSRDIYSTDYYRVGGRTMLP
IRWMPPESILYRKFTTESDVWSFGVVLWEIFTYGKQPWYQLSNTEAIDCITQGRELERPRACPP
EVYAIMRGCWQREPQQRHSIKDVHARLQALAQAPPVYLDVLG

Related Products

Product	**Cat. #**	**Size**
TRKA, GST-tag	40280	10 µg
TRKB, GST-tag	40281	10 µg
TRKC, GST-tag	40282	10 µg
TRKC (G623R) Mutant, Active)	40203	10 µg
TRKC (G623E) Mutant, Active)	40204	10 µg
TRKC (G623R L686M), Mutant, Active	40215	10 µg
TRKC (L686M), Mutant, Active	40215	10 µg
Thaw Medium 1	60187	100 ml
Growth Medium 1A	79528	500 ml

License Disclosure:
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