Human TrkA (Luc) HEK293 Reporter Cell Data Sheet

Human TrkA (Luc) HEK293 Reporter Cell

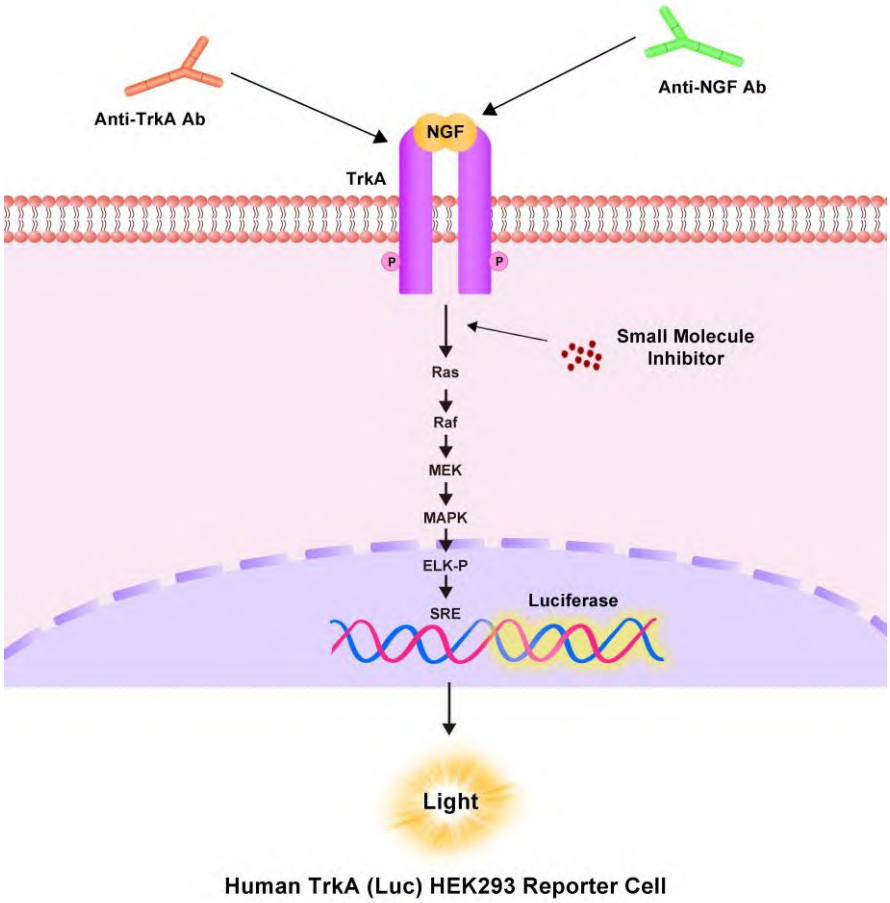
Catalog No.	Size
CHEK-ATF093	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human TrkA (Luc) HEK293 Reporter Cell was engineered to not only express SRE signaling response element, but also express the receptor full length human TrkA (Gene ID： 4914). When stimulated with human NGF protein, the NGF/TrkA interaction drives SRE-mediated luminescence. Neutralization of biological effect of human NGF protein by corresponding antibody results in a decrease in luminescence.

• *Application*

- Screen for neutralizing antibodies blocking the stimulation of human NGF protein.
- Screen for human TrkA small molecule inhibitor



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• *Cell Line Profile*

Cell line	Human TrkA (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL) + Hygromycin (20 μg/mL)
Incubation	37℃ with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

• *Materials Required for Cell Culture*

- DMEM medium (Gibco, Cat.No.11965-092)
- Fetal bovine serum (CellMax, Cat.No.SA211.02)
- Puromycin (InvivoGen, Cat.No.ant-pr-5b)
- Hygromycin B (Invitrogen, Cat.No.10687010)
- Complete Growth Medium: DMEM + 10% FBS
- Culture Medium: DMEM + 10% FBS, Puromycin (2 μg/mL), Hygromycin (20 μg/mL)
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, 430641)
- Cryogenic storage vials (SARSTEDT, 72.379.007)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Logos Biosystems, LUNA-Ⅱ)
- CO_2 Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)

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• *Recovery*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 4.0 mL complete growth medium and spin at approximately 1000 rpm for 5 minutes.
4. Resuspend cell pellet with 5 mL complete growth medium and transfer the cell suspension into T-75 flask containing 10-15 mL of pre-warmed complete growth medium.
5. Incubate at 37°C with 5% CO_2 incubator until the cells are ready to be split.

• *Subculture*

1. Remove and discard culture medium.
2. Wash the cells once with sterile PBS.
3. Add 2 mL of 0.25% trypsin to cell culture flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached.
4. Add 6.0 to 8.0 mL of culture medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessel.
6. Incubate at 37°C with 5% CO_2 incubator.

Subcultivation Ratio: A subcultivation ratio of 1:6 to 1:10 is recommended.

Medium Renewal: Every 2 to 3 days.

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• *Cryopreservation*

1. Remove and discard spent medium.
2. Detach cells from the cell culture flasks with 0.25% trypsin.
3. Centrifuge at 1000 rpm for 5 min at RT to pellet cells.
4. Resuspend the cell pellets with complete growth medium and count viable cells.
5. Centrifuge at 1000 rpm for 5 min at RT and resuspend cells in freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
6. Aliquot into cryogenic storage vials. Place vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transferring to liquid nitrogen storage.

• *Storage*

- **Product format:** Frozen
- **Storage conditions:** Liquid nitrogen immediately upon receipt

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- ***Receptor Assay***

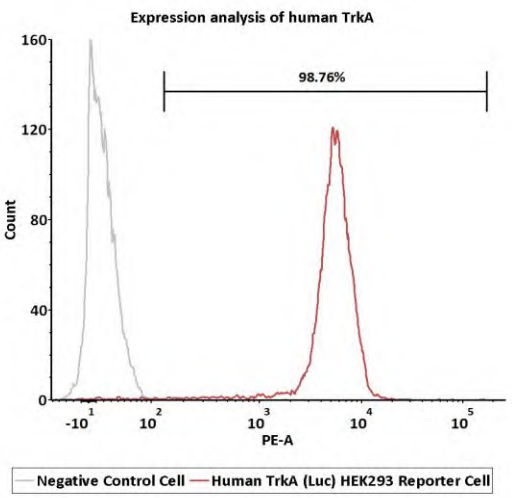

Fig1. Expression analysis of human TrkA on Human TrkA (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human TrkA (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human TrkA antibody.

- ***Signaling Bioassay***

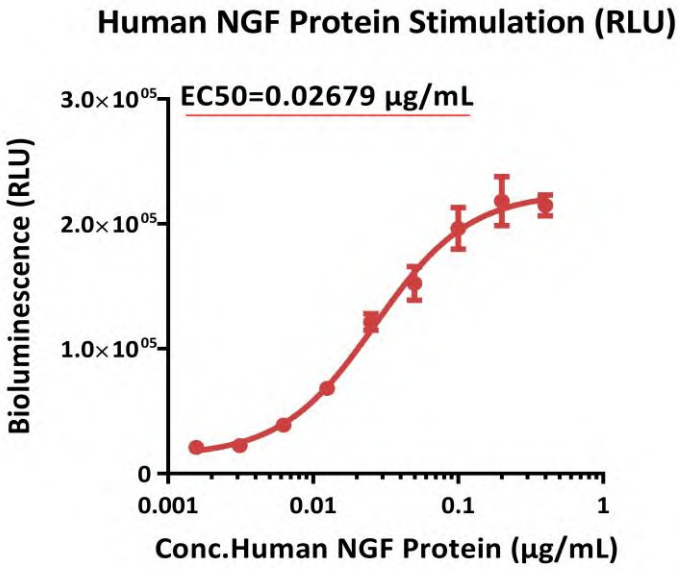

Fig2. Response to human NGF protein (RLU). The Human TrkA (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human NGF protein (Cat.No. BEF-H5214). The EC50 was approximately 0.02679 μg/mL.

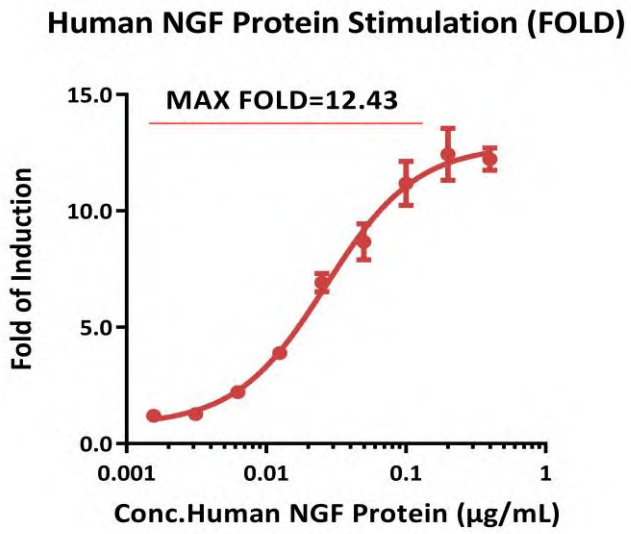

Fig3. Response to human NGF protein (FOLD). The Human TrkA (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human NGF protein (Cat.No. BEF-H5214). The max induction fold was approximately 12.43.

- ***Application***

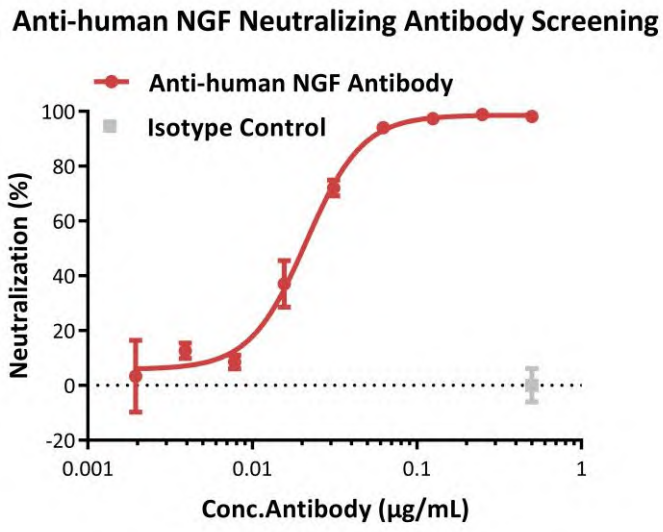

Fig4. Inhibition of human NGF protein-induced reporter activity by anti-human NGF neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human NGF protein (Cat.No. BEF-H5214) with a final concentration of 0.05 μg/mL. The EC50 of anti-human NGF neutralizing antibody is approximately 0.0212 μg/mL.

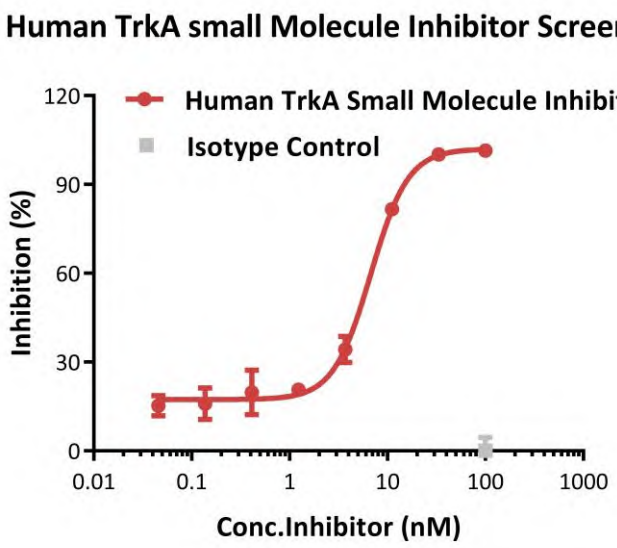

Fig5. Inhibition of human NGF protein-induced reporter activity by human TrkA small molecule inhibitor. This reporter cell was incubated with serial dilutions of inhibitors in the presence of human NGF protein (Cat.No. BEF-H5214) with a final concentration of 0.03 μg/mL. The EC50 of human TrkA small molecule inhibitor was approximately 6.725 nM.

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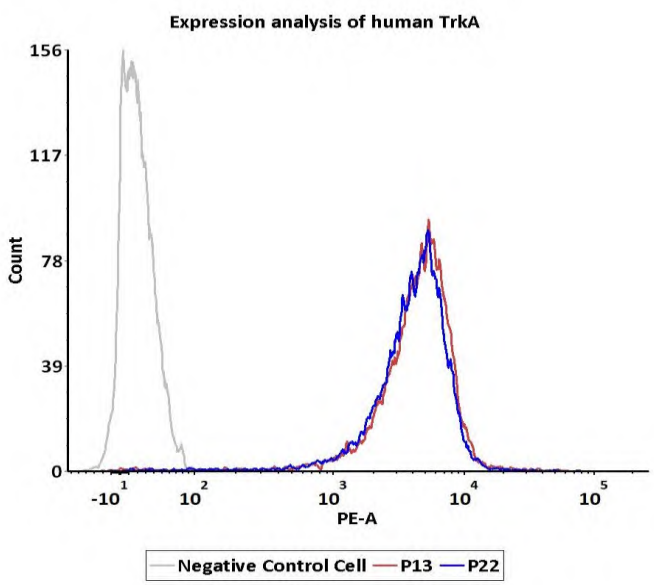
- ***Passage Stability***



Passage	MFI for TrkA (PE)
P13	4437.83
P22	4138.28

Fig6. Passage stability analysis of receptors expression by FACS. Flow cytometry surface staining of human TrkA on Human TrkA (Luc) HEK293 Reporter Cell demonstrates consistent mean fluorescent intensity across across passage 13-22.

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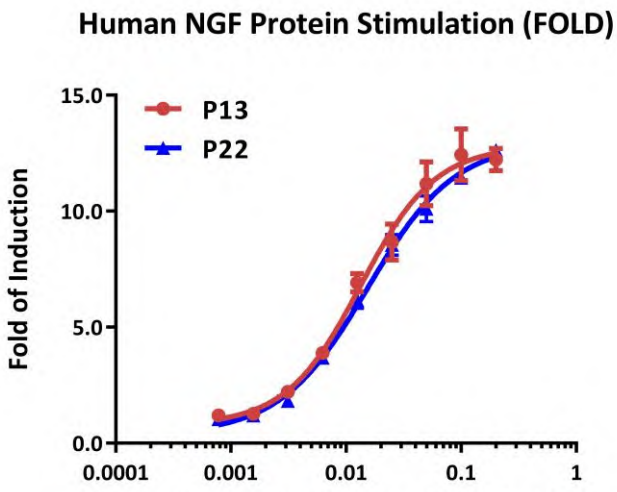

	P13	P22
EC50 (μg/mL)	0.02679	0.03079
Max Fold	12.43	12.63

Fig7. Passage stability analysis by human NGF protein stimulation. The continuously growing Human TrkA (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human NGF protein (Cat.No. BEF-H5214) stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 13-22.

• *License Disclosure*

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• *Related Products*

Products	Cat.No.
Human Beta-NGF Protein	BEF-H5214