Human TGF-beta R (Luc) HEK293 Reporter Cell Data Sheet

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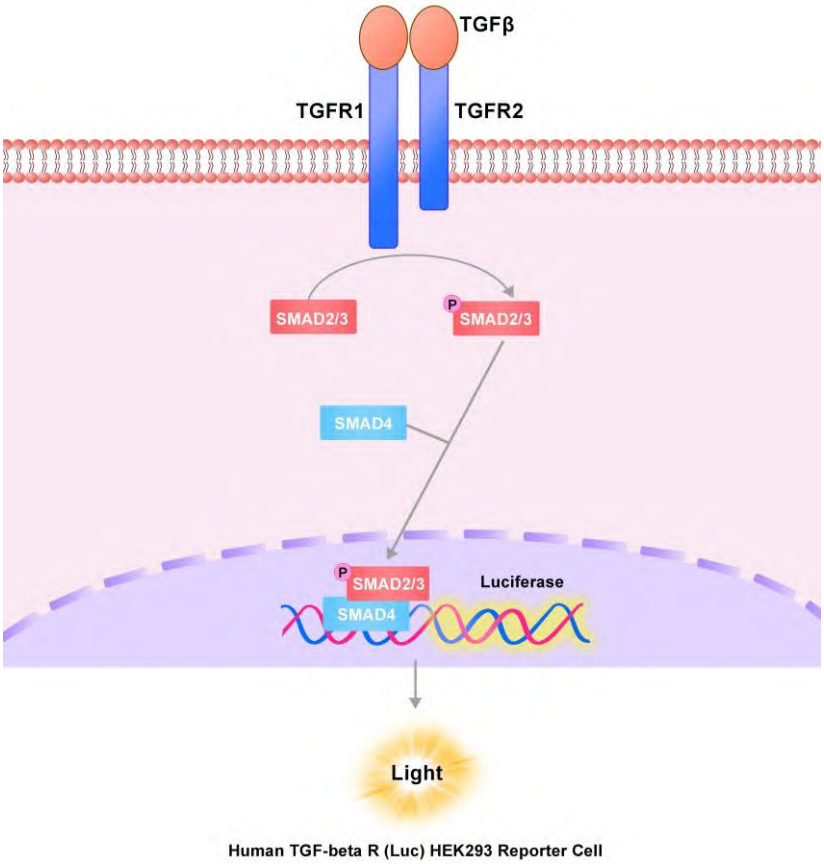
Catalog No.	Size
CHEK-ATF145	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human TGF-beta R (Luc) HEK293 Reporter Cell was engineered to express Smad signaling response element driving luciferase expressing systems. When stimulated with TGF-beta（TGF-beta 1/2/3）protein, receptor-mediated signaling can drive Smad-mediated luminescence. Neutralization of biological effect of the ligand-receptor interaction by corresponding antibody results in a decrease in luminescence.

• *Application*

- Screen for neutralizing antibodies blocking the ligand-receptor interaction.



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• *Cell Line Profile*

Cell line	Human TGF-beta R (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL)
Incubation	37°C 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)
- 0.25% Trypsin-EDTA (1X), Phenol Red (Gibco, Cat.No.25200-056)
- Penicillin-Streptomycin (Gibco, Cat.No.15140-122)
- Phosphate Buffered Saline (1X) (HyClone, Cat.No.SH30256.01)
- Complete Growth Medium: DMEM + 10% FBS
- Culture Medium: DMEM + 10% FBS, Puromycin (2 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.

• *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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• ***Signaling Bioassay***

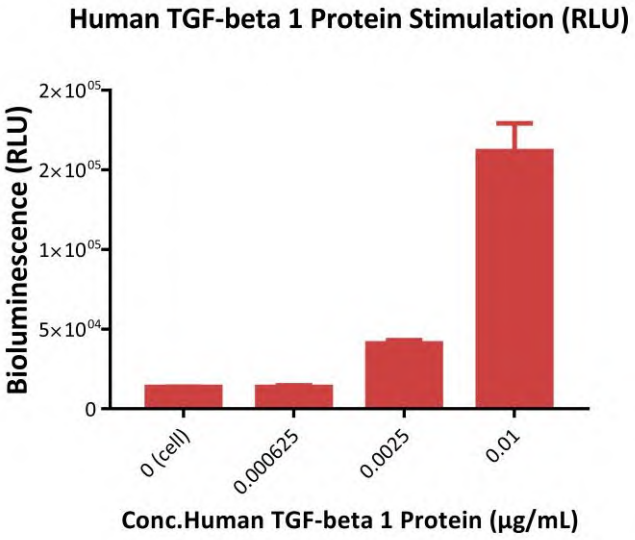

Fig1. Response to human TGF-beta 1 protein (RLU). The Human TGF-beta R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TGF-beta 1 protein (Cat.No.TG1-H4212). The max induction fold was approximately 11.81.

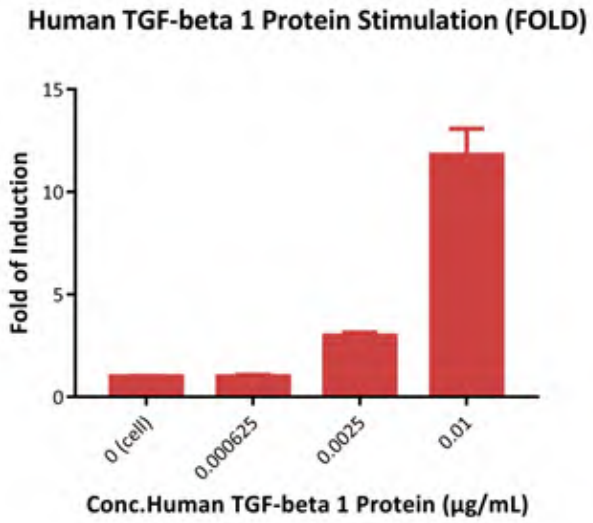

Fig2. Response to human TGF-beta 1 protein (FOLD). The Human TGF-beta R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TGF-beta 1 protein (Cat.No.TG1-H4212). The max induction fold was approximately 11.81.

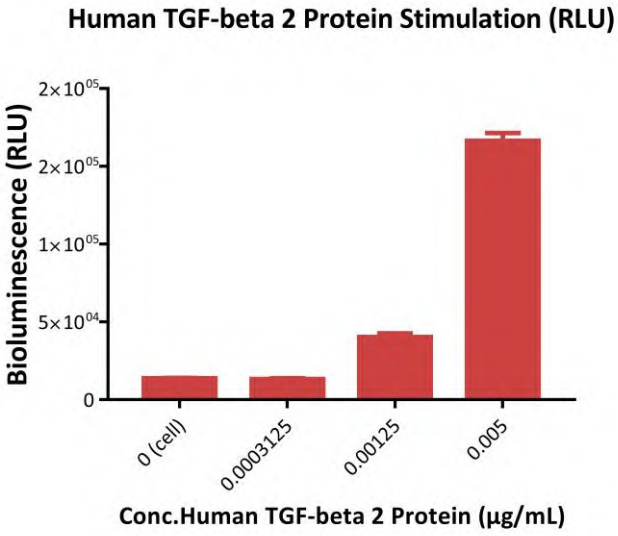

Fig3. Response to human TGF-beta 2 protein (RLU). The Human TGF-beta R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TGF-beta 2 protein (Cat.No.TG2-H4215). The max induction fold was approximately 12.15.

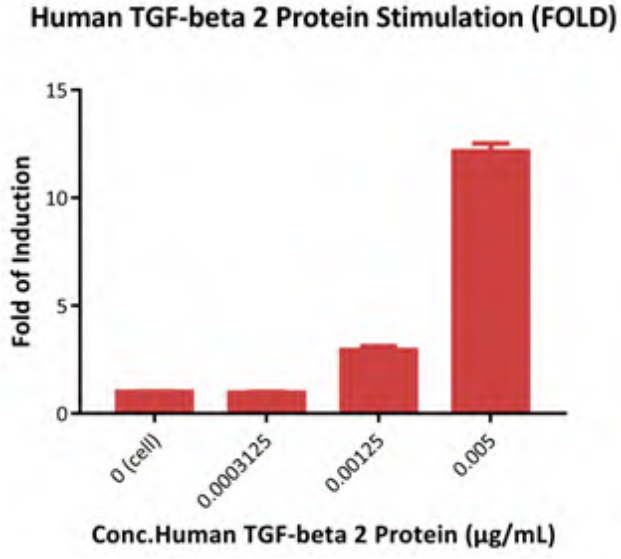

Fig4. Response to human TGF-beta 2 protein (FOLD). The Human TGF-beta R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TGF-beta 2 protein (Cat.No.TG2-H4215). The max induction fold was approximately 12.15.

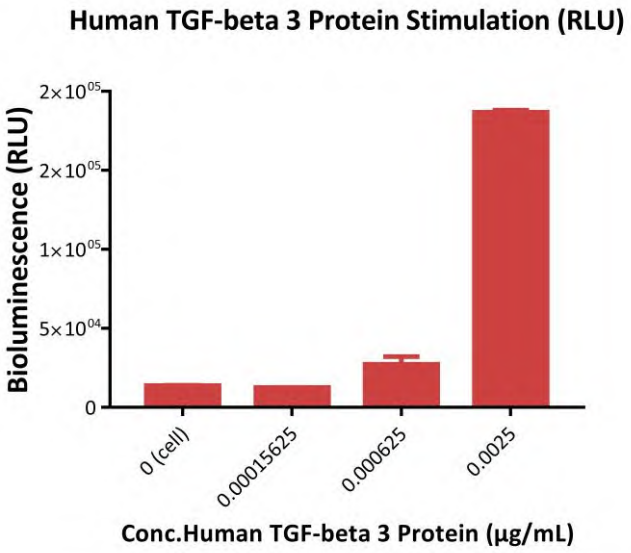

Fig5. Response to human TGF-beta 3 protein (RLU). The Human TGF-beta R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TGF-beta 3 protein (Cat.No.TG3-H5213). The max induction fold was approximately 13.64.

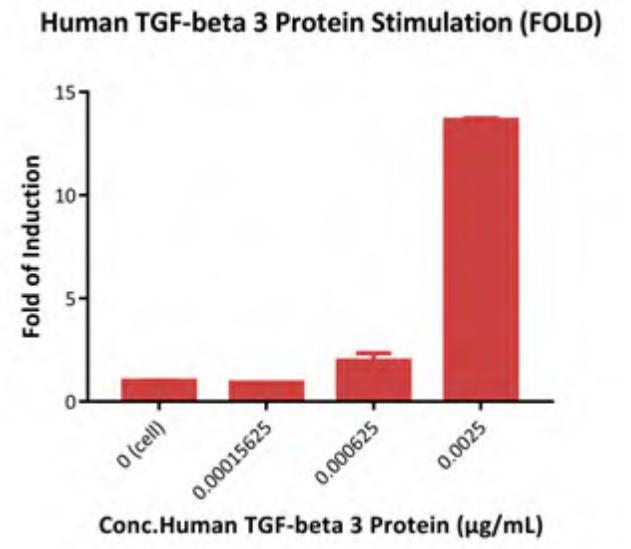

Fig6. Response to human TGF-beta 3 protein (FOLD). The Human TGF-beta R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TGF-beta 3 protein (Cat.No.TG3-H5213). The max induction fold was approximately 13.64.

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• *Application*

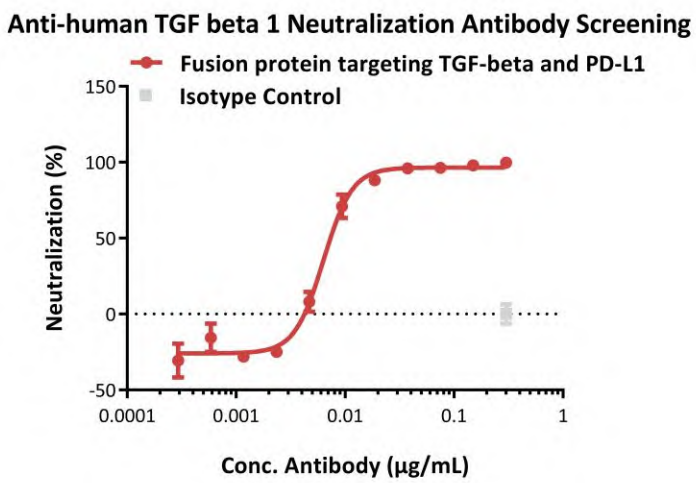

Fig7. Inhibition of human TGF-beta 1 protein protein-induced reporter activity by a bifunctional fusion protein targeting TGF-beta and PD-L1. This reporter cell was incubated with serial dilutions of the bifunctional fusion protein in the presence of human TGF-beta 1 protein (Cat.No.TG1-H4212) with a final concentration of 0.005 μg/mL. The EC50 of the bifunctional fusion protein (Bintrafusp alfa) is approximately 0.006323 μg/mL.

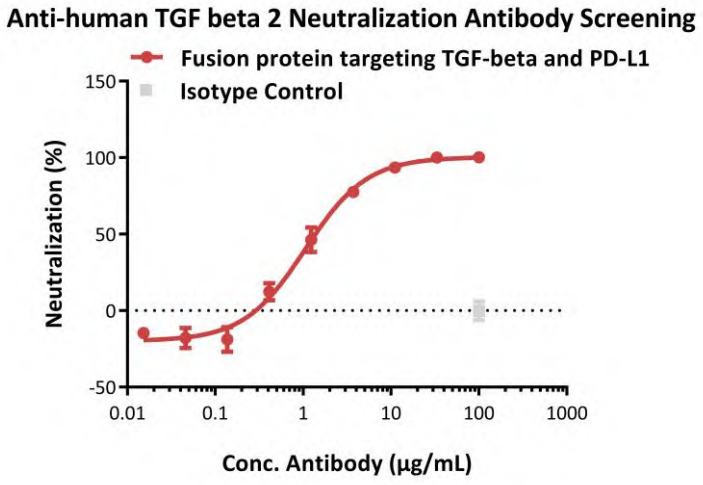

Fig8. Inhibition of human TGF-beta 2 protein protein-induced reporter activity by a bifunctional fusion protein targeting TGF-beta and PD-L1. This reporter cell was incubated with serial dilutions of the bifunctional fusion protein in the presence of human TGF-beta 2 protein (Cat.No.TG2-H4215) with a final concentration of 0.003 μg/mL. The EC50 of the bifunctional fusion protein (Bintrafusp alfa) is approximately 1.06 μg/mL.

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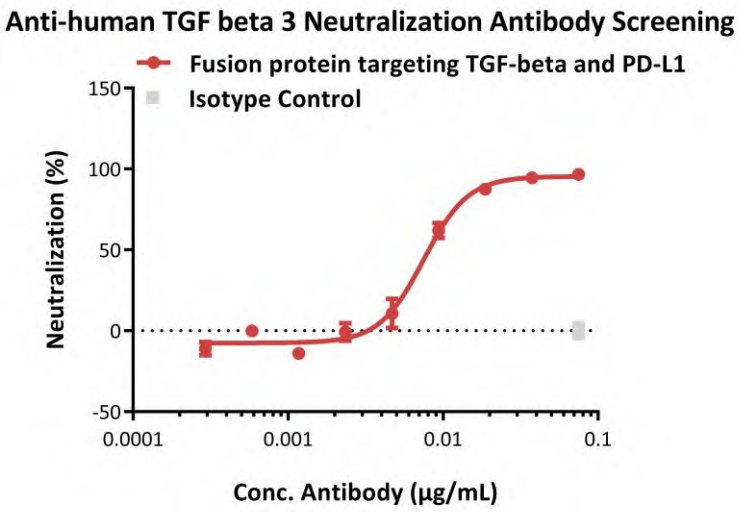

Fig9. Inhibition of human TGF-beta 3 protein protein-induced reporter activity by a bifunctional fusion protein targeting TGF-beta and PD-L1. This reporter cell was incubated with serial dilutions of the bifunctional fusion protein in the presence of human TGF-beta 3 protein (Cat.No.TG3-H5213) with a final concentration of 0.002 μg/mL. The EC50 of the bifunctional fusion protein (Bintrafusp alfa) is approximately 0.0075 μg/mL.

• *License Disclosure*

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• *Related Products*

Products	Cat.No.
Human TGF-Beta 1/TGFB1 Protein, premium grade	TG1-H4212
Human TGF-Beta 2/TGFB2 Protein, Tag Free	TG2-H4215
Human TGF-beta 3/TGFB3 Protein, premium grade	TG3-H5213