

Human Activin RII (Luc) HEK293 Reporter Cell Data Sheet

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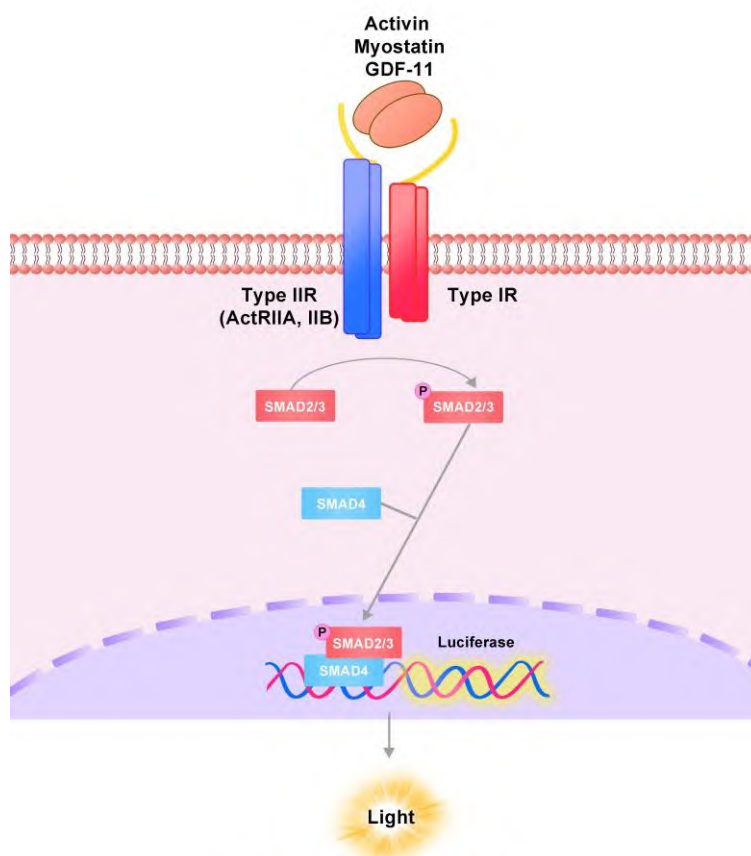
Catalog No.	Size
CHEK-ATF164	2 × (1 vial contains ~5×10 ⁶ cells)

• Description

The Human Activin RII (Luc) HEK293 Reporter Cell was engineered to express Smad signaling response element driving luciferase expressing systems. When stimulated with multiple ligands, including activin, myostatin and GDF-11, 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 Activin RII (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)
- CO₂ 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% CO₂ 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% CO₂ incubator.

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

Medium Renewal: Every 2 to 3 days.

Human Activin RII (Luc) HEK293 Reporter Cell Data Sheet

• *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*

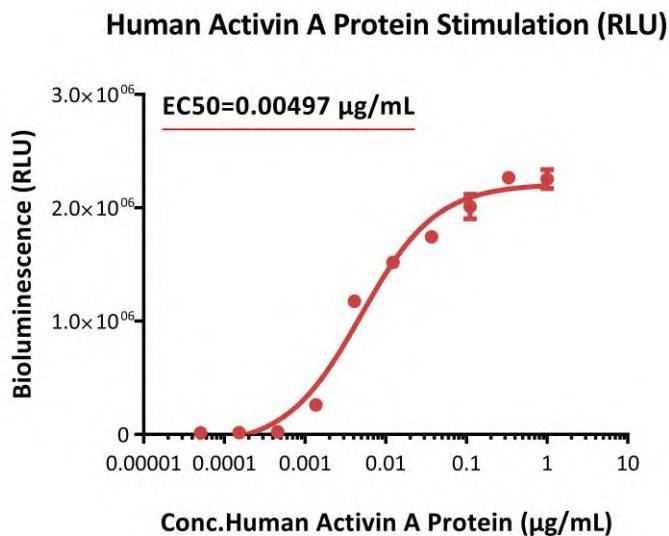


Fig1. Response to human Activin A protein (RLU). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human Activin A protein (Cat.No.ACA-H421b). The EC50 was approximately 0.00497 µg/mL.

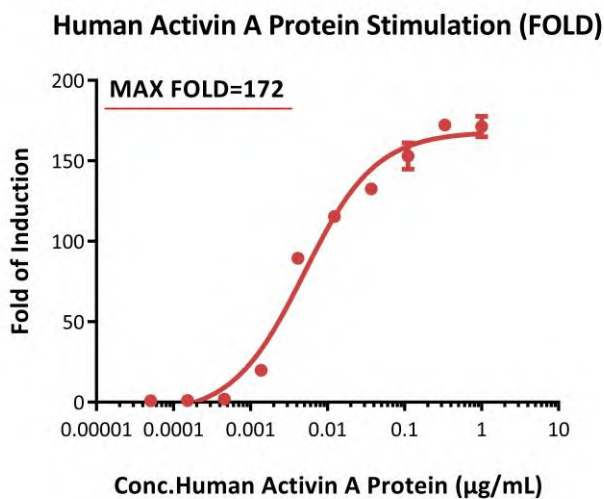


Fig2. Response to human Activin A protein (FOLD). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human Activin A protein (Cat.No.ACA-H421b). The max induction fold was approximately 172.

Human Activin RII (Luc) HEK293 Reporter Cell Data Sheet

Human GDF-11 Protein Stimulation (RLU)

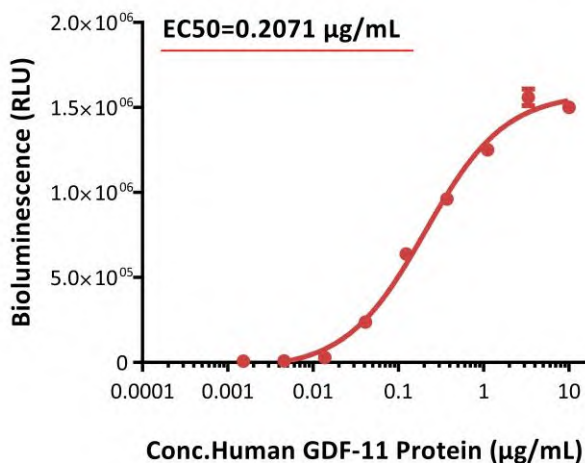


Fig3. Response to human GDF-11 protein (RLU). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDF-11 protein. The EC50 was approximately 0.2071 µg/mL.

Human GDF-11 Protein Stimulation (FOLD)

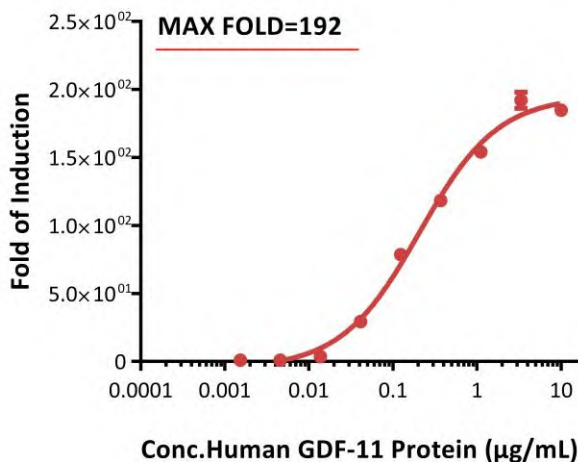


Fig4. Response to human GDF-11 protein (FOLD). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDF-11 protein (Cat.No.ACA-H421b). The max induction fold was approximately 192.

Human Activin RII (Luc) HEK293 Reporter Cell Data Sheet

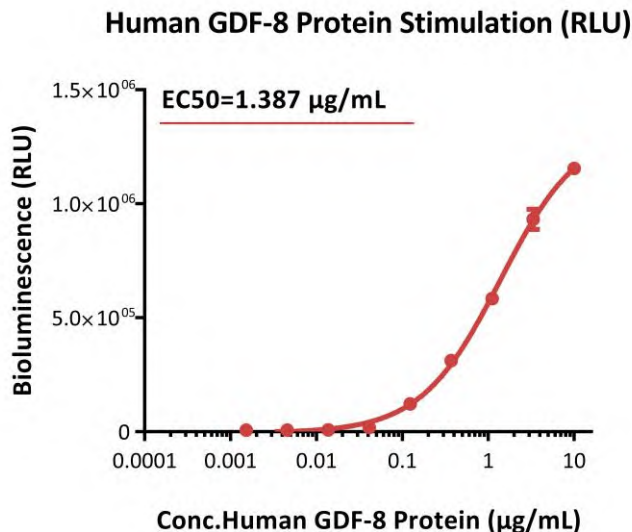


Fig5. Response to human GDF-8 protein (RLU). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDF-8 protein. The EC₅₀ was approximately 1.387 µg/mL.

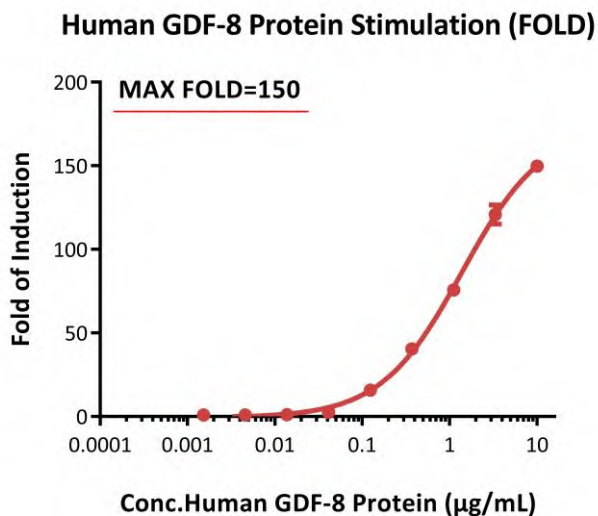


Fig6. Response to human GDF-8 protein (FOLD). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDF-8 protein. The max induction fold was approximately 150.

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• *Application*

Anti-Activin RII Neutralization Antibody Screening

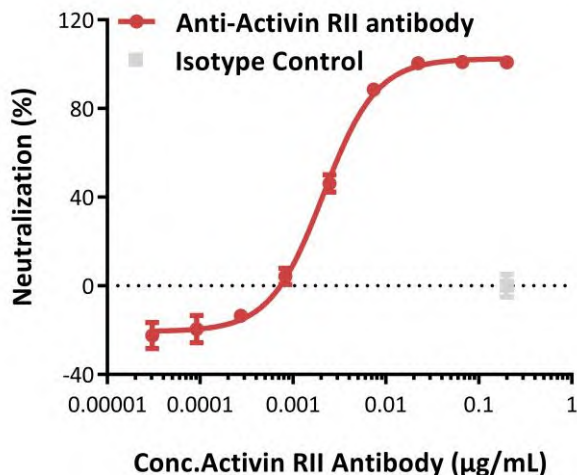


Fig7. Inhibition of human GDF-8 protein-induced reporter activity by anti-Activin RII neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human GDF-8 protein with a final concentration of 0.3 µg/mL. The EC50 of anti-Activin RII neutralizing antibody (Bimagrumab) is approximately 0.002109 µg/mL.

Anti-Activin RII Neutralization Antibody Screening

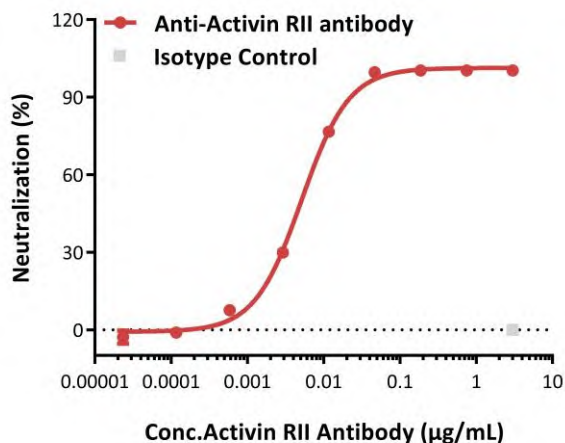


Fig8. Inhibition of human GDF-11 protein-induced reporter activity by anti-Activin RII neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human GDF-11 protein with a final concentration of 0.3 µg/mL. The EC50 of anti-Activin RII neutralizing antibody (Bimagrumab) is approximately 0.005157 µg/mL.

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Anti-Activin RII Neutralization Antibody Screening

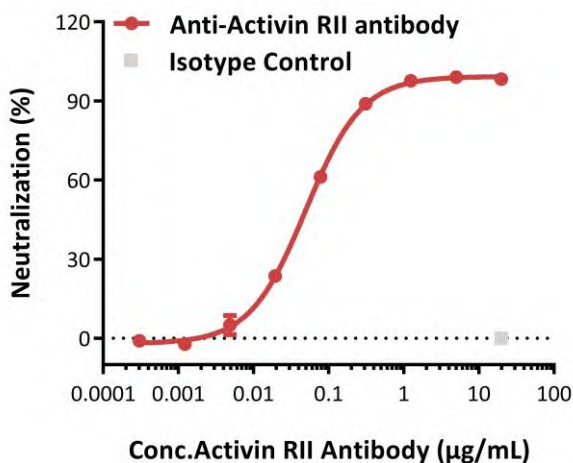


Fig9. Inhibition of human Activin A protein-induced reporter activity by anti-Activin RII neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human Activin A protein (Cat.No.ACA-H421b) with a final concentration of 0.01 µg/mL. The EC50 of anti-Activin RII neutralizing antibody (Bimagrumab) is approximately 0.04982 µg/mL.

• License Disclosure

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• Related Products

Products

Human Activin A / INHBA Protein, premium grade

Cat.No.

ACA-H421b