

# Human Activin RII (Luc) HEK293 Reporter Cell Data Sheet

## Human Activin RII (Luc) HEK293 Reporter Cell

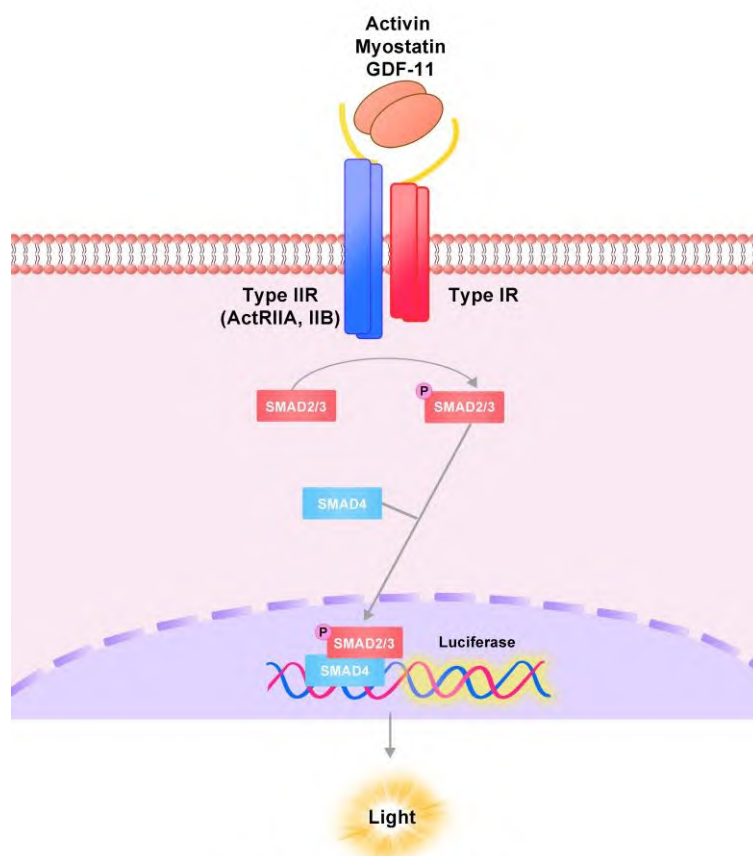
Catalog No.	Size
CHEK-ATF164	2 × (1 vial contains ~5×10 <sup>6</sup> cells)

### • 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

<b>Cell line</b>	Human Activin RII (Luc) HEK293 Reporter Cell
<b>Host Cell</b>	HEK293
<b>Property</b>	Adherent
<b>Complete Growth Medium</b>	DMEM + 10% FBS
<b>Selection Marker</b>	Puromycin (2 µg/mL)
<b>Incubation</b>	37°C with 5% CO <sub>2</sub>
<b>Doubling Time</b>	22-24 hours
<b>Transduction Technique</b>	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, 1%P/S
- Culture Medium: DMEM + 10% FBS, Puromycin (2 µg/mL), 1%P/S
- 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> incubator.

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

**Medium Renewal:** Every 2 to 3 days.

**Note:** After recovery for 1-2 generations with the complete growth medium not containing the selection marker, if the cell state is well, changing to the culture medium containing the selection marker.

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## • *Cryopreservation*

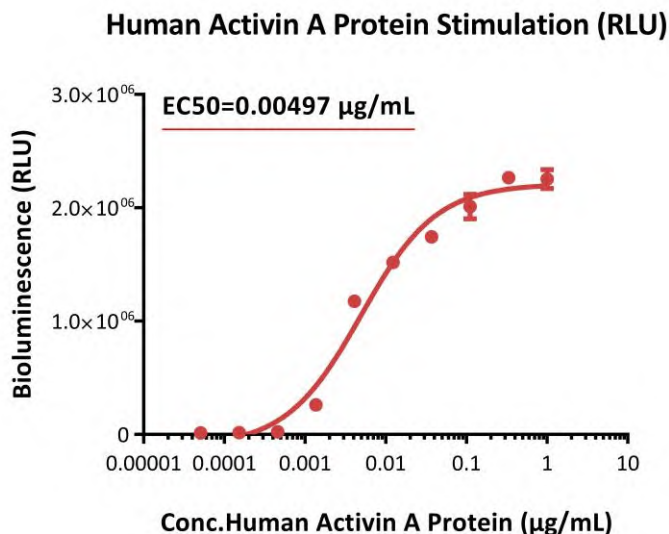
1. Remove and discard spent medium.
2. Detach cells from the cell culture flasks with 0.25% trypsin.
3. Centrifuge at 1000 rpm for 5 min at RT to pellet cells.
4. Resuspend the cell pellets with complete growth medium and count viable cells.
5. Centrifuge at 1000 rpm for 5 min at RT and resuspend cells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

## • *Storage*

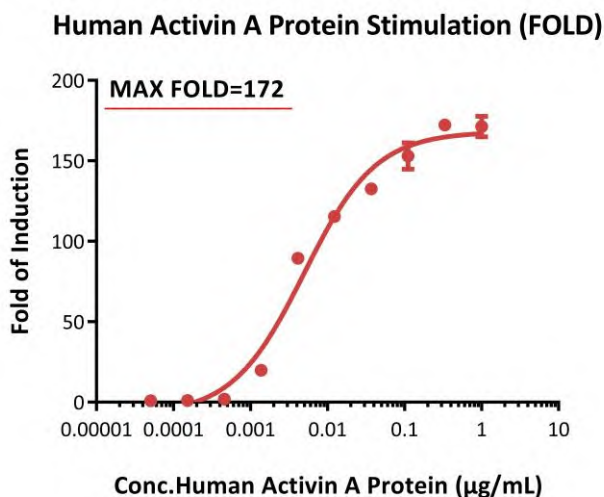
- **Product format:** Frozen
- **Storage conditions:** Liquid nitrogen immediately upon receipt

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• *Signaling Bioassay*



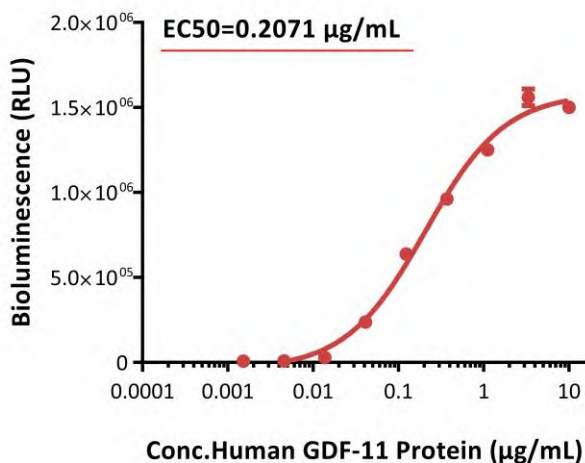
**Fig1. Response to human Activin A protein (RLU).** The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human Activin A protein (Cat.No.ACA-H421b). The EC50 was approximately 0.00497 µg/mL.



**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.

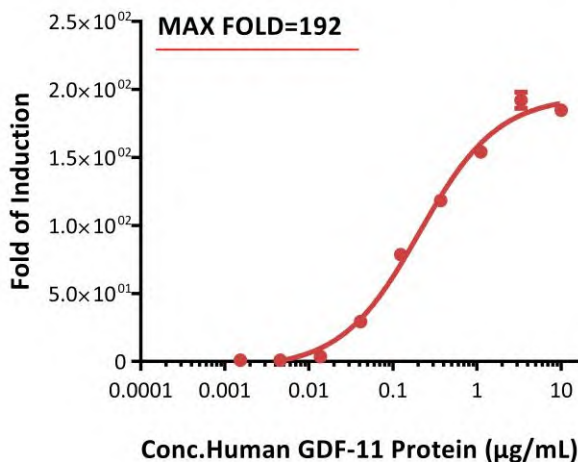
# Human Activin RII (Luc) HEK293 Reporter Cell Data Sheet

## Human GDF-11 Protein Stimulation (RLU)



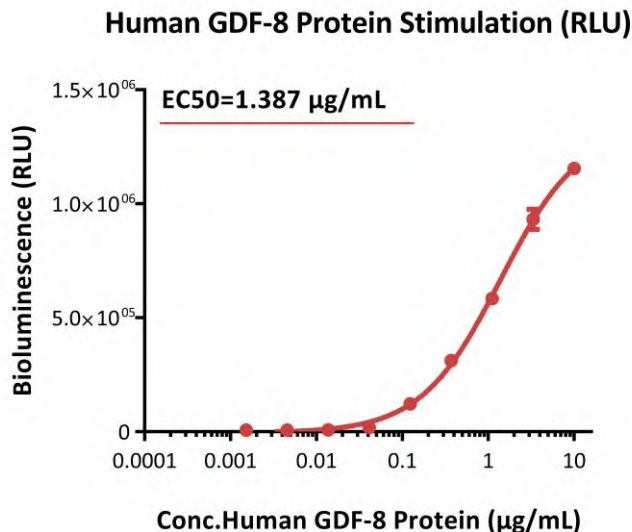
**Fig3. Response to human GDF-11 protein (RLU).** The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDF-11 protein. The EC50 was approximately 0.2071 µg/mL.

## Human GDF-11 Protein Stimulation (FOLD)

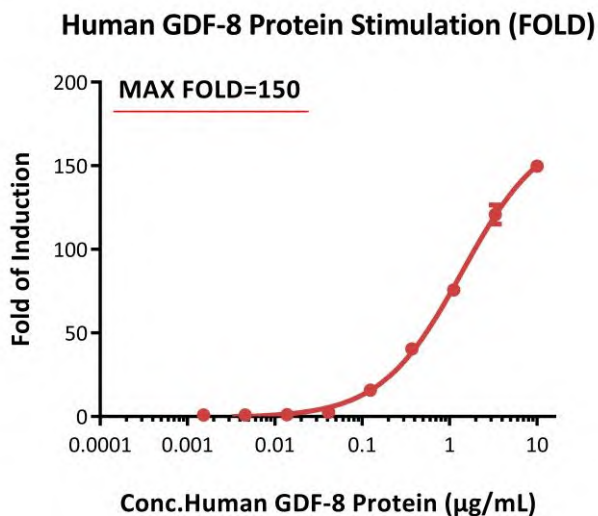


**Fig4. Response to human GDF-11 protein (FOLD).** The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDF-11 protein. The max induction fold was approximately 192.

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**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  $EC_{50}$  was approximately  $1.387 \mu\text{g/mL}$ .

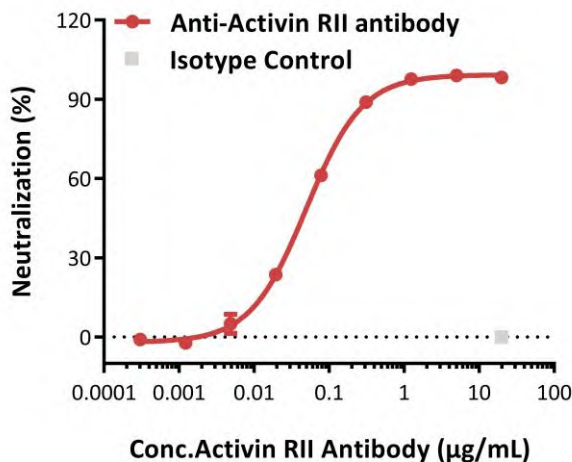


**Fig6. Response to human GDF-8 protein (FOLD).** The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDF-8 protein. The max induction fold was approximately 150.

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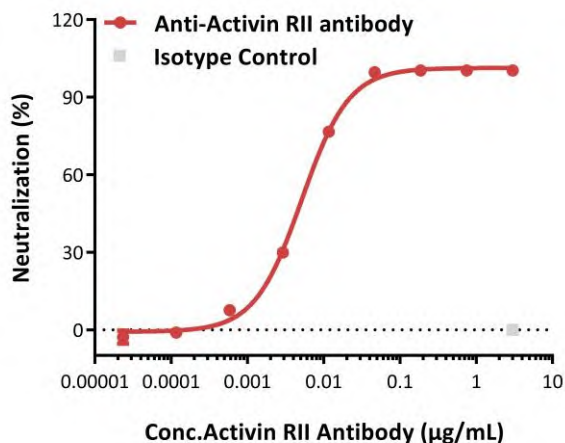
• *Application*

## Anti-Activin RII Neutralization Antibody Screening



**Fig7. Inhibition of human 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.

## Anti-Activin RII Neutralization Antibody Screening

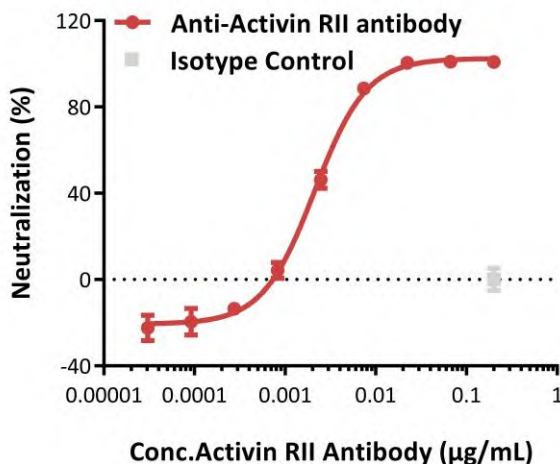


**Fig8. Inhibition of human GDF-11 protein-induced reporter activity by anti-Activin RII neutralizing antibody.** This reporter cell was incubated with serial dilutions of antibodies in the presence of human GDF-11 protein with a final concentration of 0.3 µg/mL. The EC50 of anti-Activin RII neutralizing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



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## Anti-Activin RII Neutralization Antibody Screening



**Fig9. Inhibition of human 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.

### • License Disclosure

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### • Related Products

#### Products

Human Activin A / INHBA Protein, premium grade

#### Cat.No.

ACA-H421b