Human Activin RII (Luc) HEK293 Reporter Cell Data Sheet

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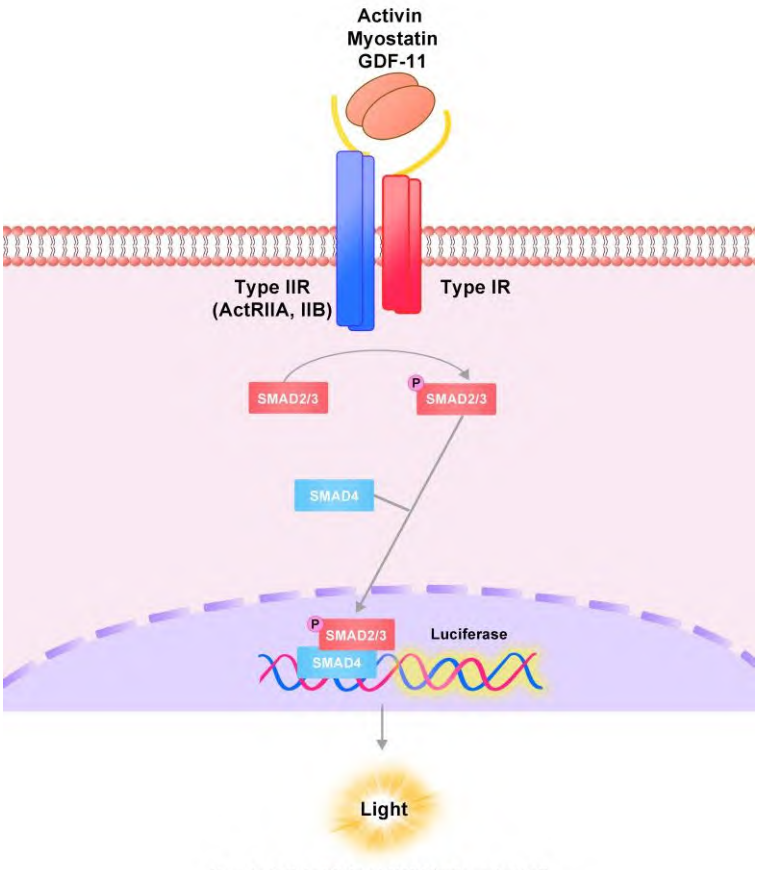
Catalog No.	Size
CHEK-ATF164	2 × (1 vial contains ~5×10^6 cells)

• *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 (BasalMedia, Cat. No. L120KJ)

Note: If you are unable to obtain the specified DMEM medium (BasalMedia, Cat. No. L120KJ) in China, you may use an alternative DMEM medium (Gibco, Cat. No. 11965-092) or another suitable medium for culturing.

- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Puromycin (InvivoGen, Cat. No. ant-pr-5b)

Note: For selection antibiotics, we highly recommend using the specified brand. The activity of antibiotics may vary between manufacturers, so if you choose to use a different brand, it is essential to validate whether the concentration recommended in the culture medium is suitable. Regardless of the brand used, we recommend maintaining a backup culture without selection antibiotics to avoid potential cell loss due to inappropriate antibiotic concentration.

- 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, Cat. No. 430641)
- Cryogenic storage vials (SARSTEDT, Cat. No. 72.379.007)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell200A Plus)
- CO_2 Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

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• *Recovery*

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize the risk of contamination, ensure the cap remains out of the water. Thawing should be completed quickly, typically within 3-5 minutes.
2. After thawing, promptly remove the vial from the water bath and decontaminate it by spraying with 70% ethanol. From this point onward, all operations must be performed under strict aseptic conditions.
3. Transfer the contents of the vial to a centrifuge tube containing 4.0 mL of complete growth medium. Centrifuge at approximately 1000 rpm for 5 minutes.
4. Resuspend the cell pellet with 5 mL complete growth medium and transfer the cell suspension into a 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. Cell viability may be low after thawing, and full recovery may take up to a week. Monitor the cells daily until the culture reaches 80-90% confluency. At this point, remove and discard the spent medium. Avoid allowing the cells to become over-confluent to ensure optimal cell health.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of culture medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps.
5. Transfer appropriate aliquots of the cell suspension to a new T-75 flask. A subcultivation ratio of 1:4 to 1:8 is recommended. Adjust the ratio based on your specific culture system.
6. Incubate at 37°C with 5% CO_2 incubator.
7. When the cell culture reaches 80-90% confluency, proceed to the next subculture. Avoid over-confluency, as this may negatively impact cell performance in subsequent passages.

Note: After recovery, maintain the cells for 1-2 passages in the complete growth medium not containing the selection marker, if the cells are in good condition, transition to the culture medium containing the selection marker during subculturing.

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• *Cryopreservation*

1. When the cell culture reaches 80-90% confluency, remove and discard the spent medium.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of complete growth medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps. Count the viable cells.
5. Transfer the cell suspension to a centrifuge tube. Centrifuge at 1000 rpm for 5 min at room temperature to pellet the cells.
6. After centrifugation, discard the supernatant. Resuspend the cells in ice cold freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
7. Aliquot the cell suspension into cryogenic storage vials. Place the vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transfer to liquid nitrogen storage for long-term storage.

Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *Storage Condition*

Cells must be received in a frozen state on dry ice and should be transferred to liquid nitrogen or a –80°C freezer immediately upon receipt. If stored in a –80°C freezer, it is recommended to limit the storage period to no more than two weeks. For long-term preservation, transfer the cells to liquid nitrogen is highly recommended.

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- ***Signaling Bioassay***

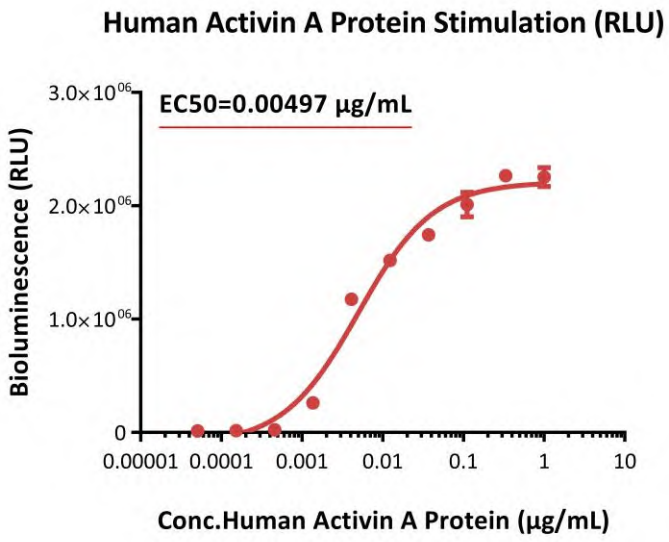

Fig1. Response to human Activin A protein (RLU). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human Activin A protein (Cat. No. ACA-H421b). The EC50 was approximately 0.00497 μg/mL.

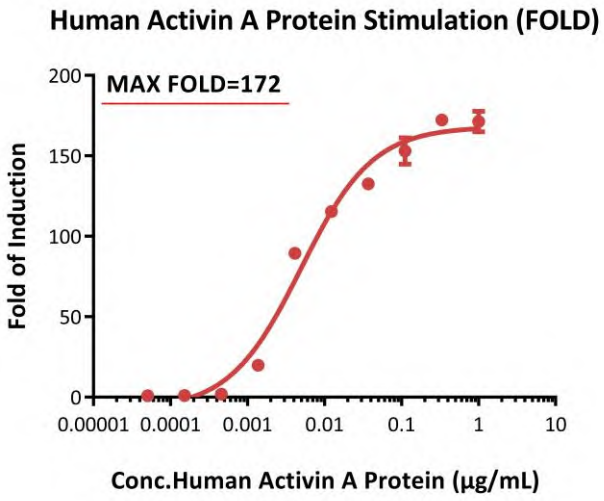

Fig2. Response to human Activin A protein (FOLD). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human Activin A protein (Cat. No. ACA-H421b). The max induction fold was approximately 172.

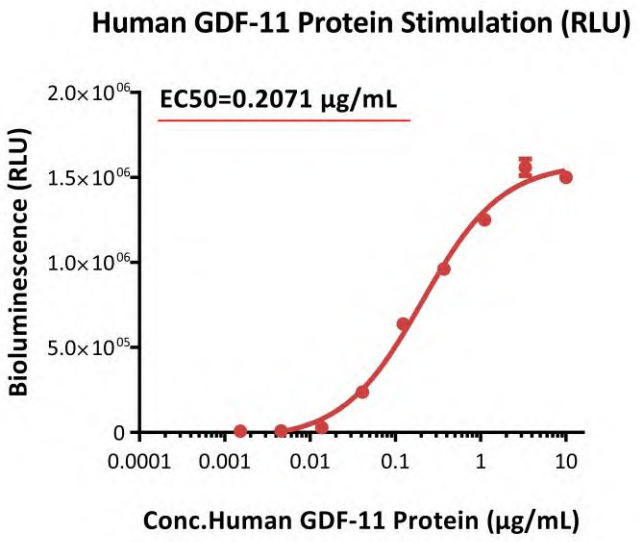

Fig3. Response to human GDF-11 protein (RLU). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDF-11 protein. The EC50 was approximately 0.2071 μg/mL.

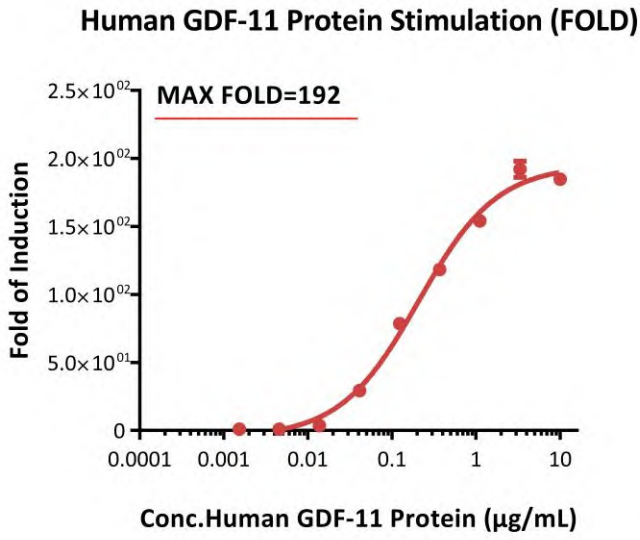

Fig4. Response to human GDF-11 protein (FOLD). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDF-11 protein. The max induction fold was approximately 192.

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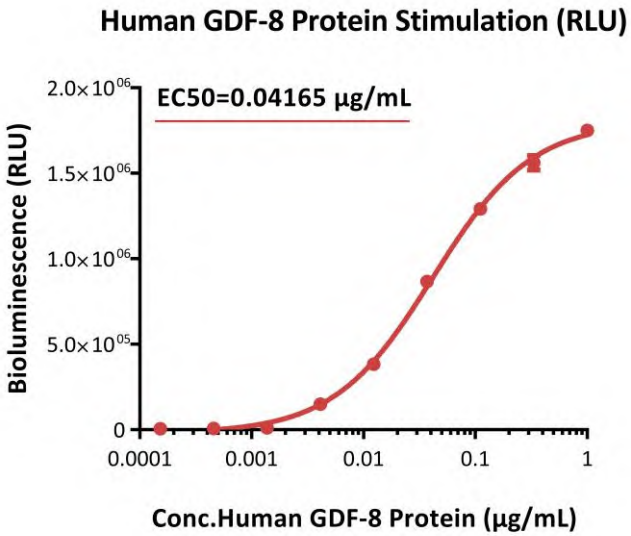

Fig5. Response to human GDF-8 protein (RLU). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDF-8 protein (Cat. No. GD8-H5344). The EC50 was approximately 0.04165 μg/mL.

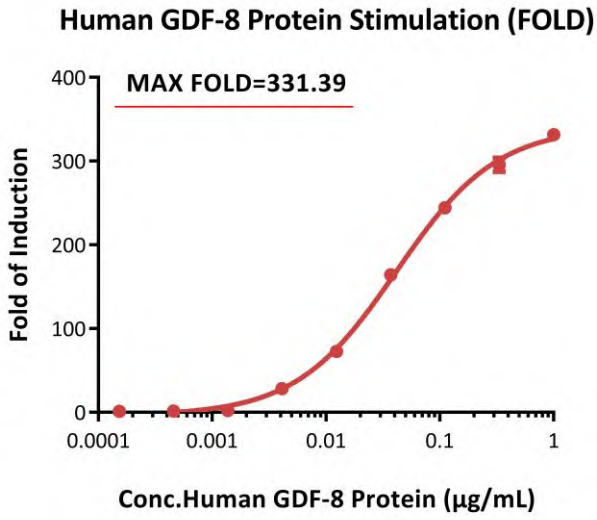

Fig6. Response to human GDF-8 protein (FOLD). The Human Activin RII (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDF-8 protein (Cat. No. GD8-H5344). The max induction fold was approximately 331.39.

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• ***Application***

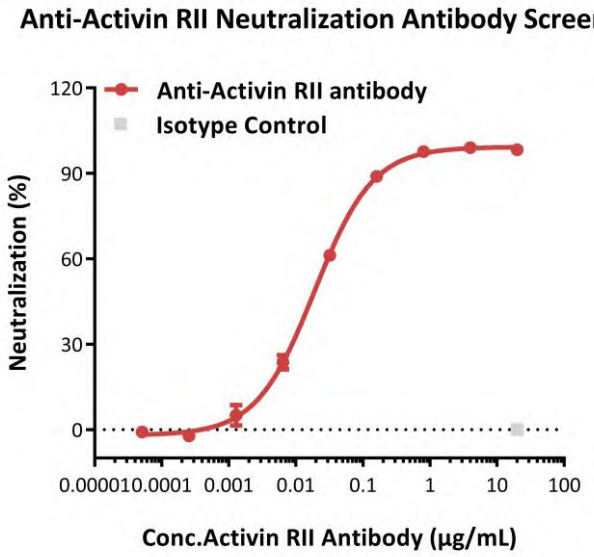

Fig7. Inhibition of human Activin A protein-induced reporter activity by anti-Activin RII neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human Activin A protein (Cat. No. ACA-H421b) with a final concentration of 0.01 μg/mL. The EC50 of anti-Activin RII neutralizing antibody (Bimagrumab) is approximately 0.01898 μg/mL.

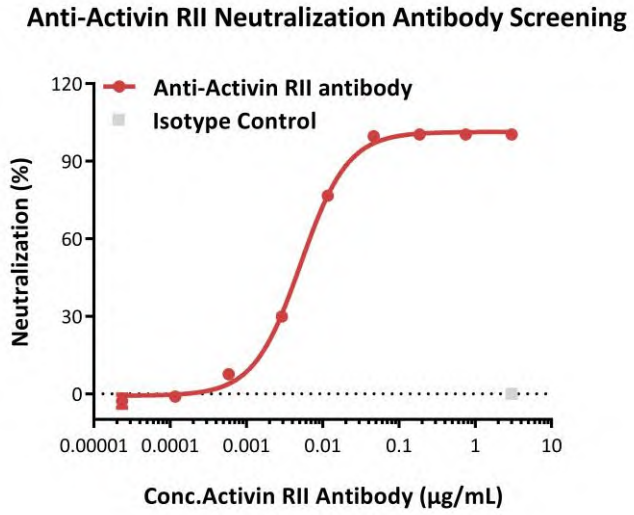

Fig8. Inhibition of human GDF-11 protein-induced reporter activity by anti-Activin RII neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human GDF-11 protein with a final concentration of 0.3 μg/mL. The EC50 of anti-Activin RII neutralizing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

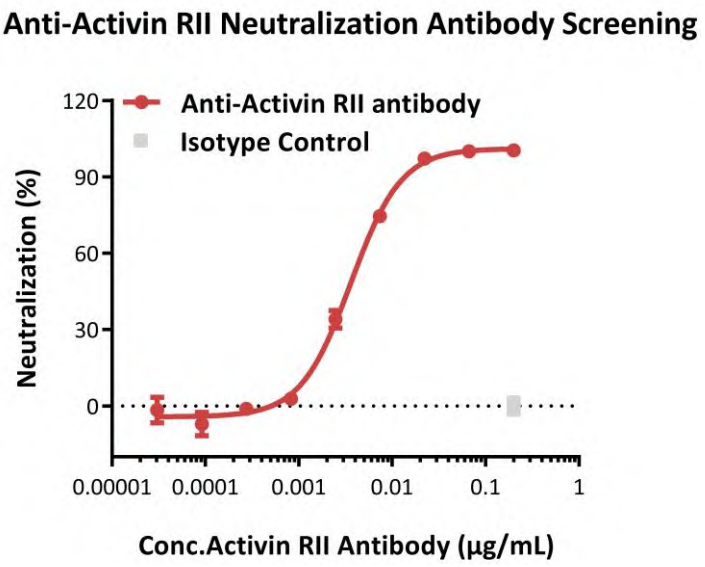

Fig9. Inhibition of human GDF-8 protein-induced reporter activity by anti-Activin RII neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human GDF-8 protein (Cat. No. GD8-H5344) with a final concentration of 0.01 μg/mL. The EC50 of anti-Activin RII neutralizing antibody (Bimagrumab) is approximately 0.003617 μg/mL.

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• *Related Products*

Products	Cat.No.
Human Activin A / INHBA Protein, premium grade	ACA-H421b
Human GDF-8 protein	GD8-H5344
Human GLP-1R (Luc) HEK293 Reporter Cell	CHEK-ATF096
Human GCGR (Luc) HEK293 Reporter Cell	CHEK-ATF103
Human GIPR (Luc) HEK293 Reporter Cell	CHEK-ATF104
HEK293/Human GLP-1R Stable Cell Line (High Expression)	CHEK-ATP160
HEK293/Human GLP-1R Stable Cell Line (Medium Expression)	CHEK-ATP161
HEK293/Human GLP-1R Stable Cell Line (Low Expression)	CHEK-ATP162
Human FGF-21 (Luc) HEK293 Reporter Cell	CHEK-ATF163
HEK293/Human GPR75 Stable Cell Line	CHEK-ATP174
Human THRA (Luc) HEK293 Reporter Cell	CHEK-ATF180
Human THRB (Luc) HEK293 Reporter Cell	CHEK-ATF181
HEK293/Human GLP-1R&GIPR Stable Cell Line	CHEK-ATP205
HEK293/Human GIPR Stable Cell Line (High Expression)	CHEK-ATP206
HEK293/Human GIPR Stable Cell Line (Medium Expression)	CHEK-ATP207
HEK293/Human GIPR Stable Cell Line (Low Expression)	CHEK-ATP208
HEK293/Human GCGR Stable Cell Line (High Expression)	CHEK-ATP209
HEK293/Human GCGR Stable Cell Line (Medium Expression)	CHEK-ATP210
HEK293/Human GCGR Stable Cell Line (Medium Expression)	CHEK-ATP210
HEK293/Human GCGR Stable Cell Line (Low Expression)	CHEK-ATP211
HEK293/Human ASGR1&ASGR2 Stable Cell Line	CHEK-ATP172
HEK293/Human ASGR1 Stable Cell Line	CHEK-ATP080
HEK293/Human LDL R Stable Cell Line	CHEK-ATP158