

Human IGF-1 R (Luc) HEK293 Reporter Cell

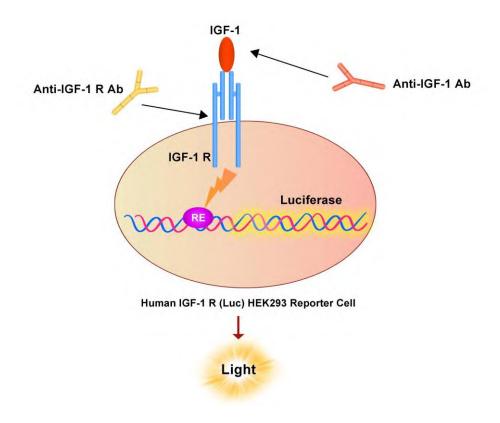
Catalog No.	Size
CHEK-ATF107	$2 \times (1 \text{ vial contains } \sim 5 \times 10^{6} \text{ cells})$

• Description

The Human IGF-1 R (Luc) HEK293 Reporter Cell was engineered to not only express signaling response element, but also express the receptor full length human IGF-1 R (Gene ID: 3480). When stimulated with human IGF-1 protein, the IGF-1/IGF-1 R interaction drives RE-mediated luminescence. Neutralization of biological effect of human IGF-1 protein by corresponding antibody results in a decrease in luminescence.

• Application

Screen for neutralizing antibodies blocking the stimulation of human IGF-1 protein.





• Cell Line Profile

Cell line	
Host Cell	
Property	
Complete Growth Medium	
Selection Marker	
Incubation	
Doubling Time	
Transduction Technique	

Human ICF-1 R (Luc) HEK293 Reporter Cell HEK293 Adherent DMEM + 10% FBS Puromycin (2 µg/mL) + Hygromycin (20 µg/mL) 37°C with 5% CO₂ 22-24 hours 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)
- CO₂ Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)



• 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 $^{\circ}$ 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.



• 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 $a -80^{\circ}C$ freezer overnight, then transferring to liquid nitrogen storage.
- Storage
 - **Product format:** Frozen
 - Storage conditions: Liquid nitrogen immediately upon receipt



• Receptor Assay

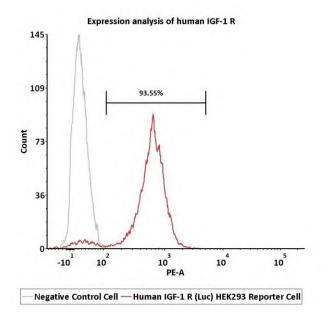


Fig1. Expression analysis of human IGF-1 R on Human IGF-1 R (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human IGF-1 R (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human IGF-1 R antibody.



• Signaling Bioassay

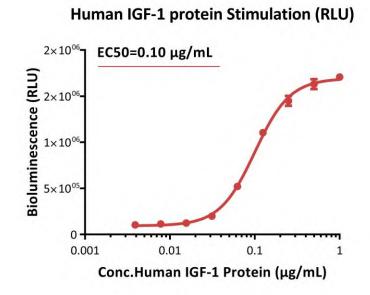


Fig2. Response to human IGF-1 protein (RLU). This reporter cell was incubated with serial dilutions of human IGF-1 protein (Cat.No.IG1-H5245). The EC50 was approximately 0.10 µg/mL.

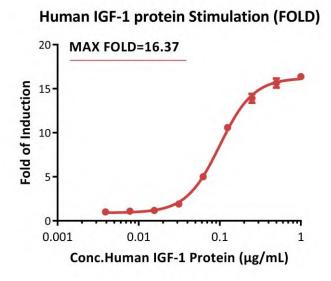


Fig3. Response to human IGF-1 protein (FOLD). This reporter cell was incubated with serial dilutions of human IGF-1 protein (Cat.No.IG1-H5245). The max induction fold was approximately 16.37.



• Application



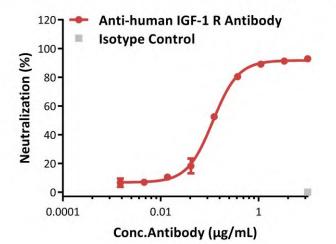


Fig4. Inhibition of human IGF-1 protein-induced reporter activity. This reporter cell was incubated with serial dilutions of antibodies in the presence of human IGF-1 protein (Cat.No.IG1-H5245) with a final concentration of 0.1 µg/mL. The EC50 of anti-human IGF-1 R neutralizing antibody (Teprotumumab) is approximately 0.12 µg/mL.



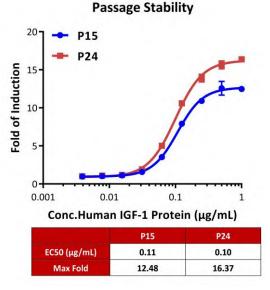


Fig5. Passage stability analysis by Signaling Bioassay. The continuously growing Human IGF-1 R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IGF-1 protein (Cat.No.IG1-H5245). Human IGF-1 protein stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 15-24.



• License Disclosure

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• Related Products

Products

Human IGF-I Protein, His Tag

<u>Cat.No.</u> IG1-H5245