Human EGF R (Luc) HEK293 Reporter Cell Data Sheet

Human EGF R (Luc) HEK293 Reporter Cell

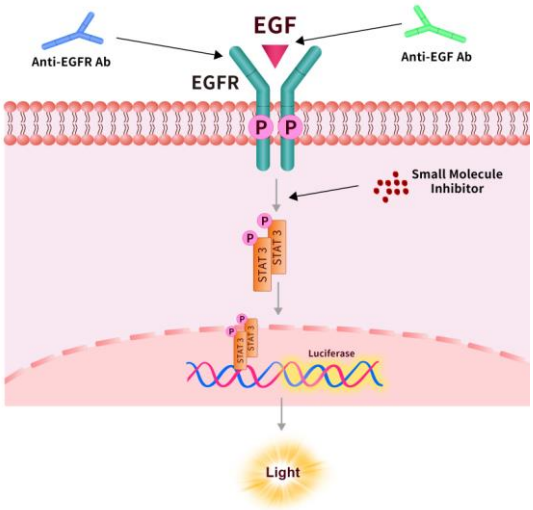
Catalog No.	Size
CHEK-ATF049	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human EGF R (Luc) HEK293 Reporter Cell was engineered to not only express STAT3 signaling response element, but also express the receptor full length human EGF R (Gene ID: 1956). When stimulated with human EGF protein, the EGF/EGF R interaction drives STAT3-mediated luminescence. Inhibition of EGF binding to EGF R by either anti-EGF or anti-EGF R antibodies results in a decrease in luminescence.

• *Application*

- Screen for anti-human EGF R or anti-human EGF neutralizing antibody.
- Screen for human EGF R small molecule inhibitor



• *Cell Line Profile*

Cell line	Human EGF R (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Hygromycin (40 μg/mL) + Puromycin (2 μg/mL)
Incubation	37℃ with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

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• *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, Hygromycin (40 μg/mL), 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)

• *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.

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• *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℃ 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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- ***Receptor Assay***

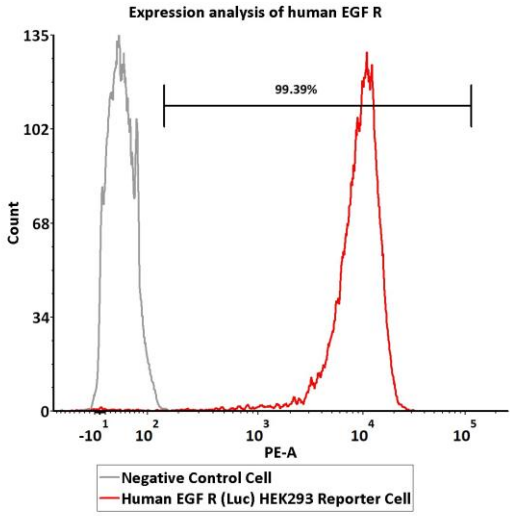

Fig1. Expression analysis of human EGF R on Human EGF R (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human EGF R (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-EGF R antibody.

- ***Signaling Bioassay***

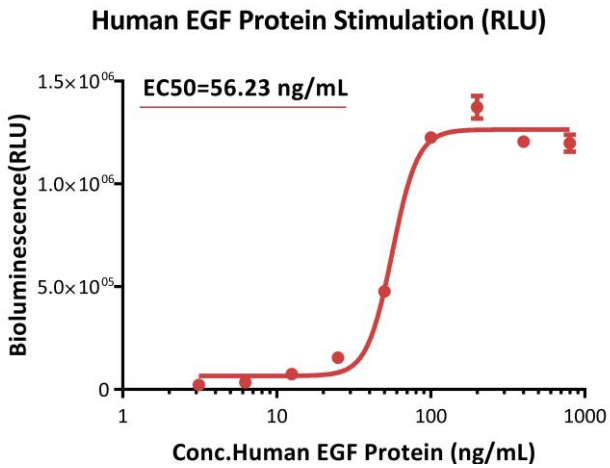

Fig2. Response to human EGF protein (RLU). The Human EGF R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human EGF protein (Cat.No.EGF-H52H3). The EC50 was approximately 56.23 ng/mL.

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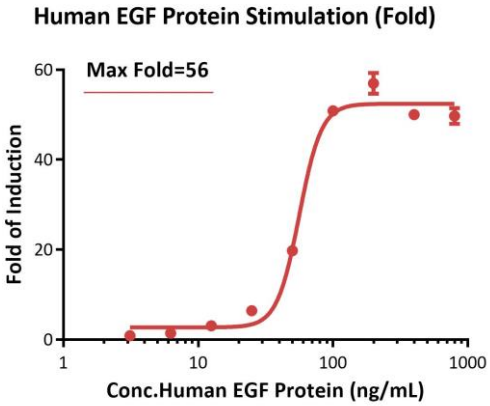

Fig3. Response to human EGF protein (Fold). The Human EGF R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human EGF protein (Cat.No.EGF-H52H3). The max induction fold was approximately 56.

- ***Application***

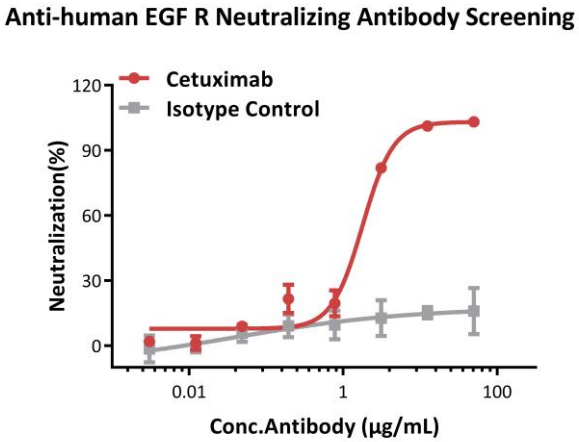

Fig4. Inhibition of human EGF protein-induced reporter activity by anti-human EGF R neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human EGF protein (Cat.No.EGF-H52H3) with a final concentration of 50 ng/mL. The EC50 of anti-human EGF R neutralizing antibody (Cetuximab) is approximately 1.793 μg/mL.

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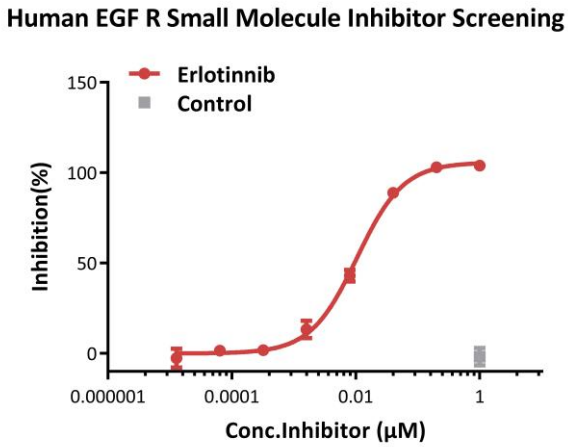
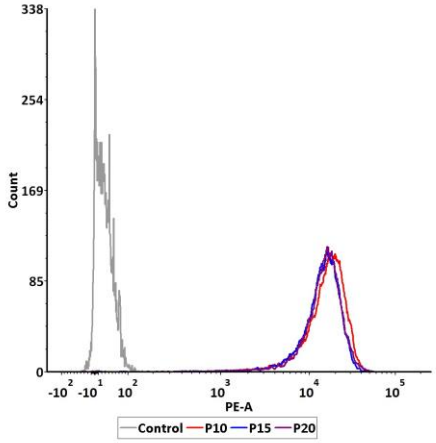


Fig5. Inhibition of human EGF protein-induced reporter activity by human EGF R small molecule inhibitor. This reporter cell was incubated with serial dilutions of inhibitors in the presence of human EGF protein (Cat.No.EGF-H52H3) with a final concentration of 50 ng/mL. The EC50 of human EGF R small molecule inhibitor (Erlotinib) was approximately 0.01 μM.

- ***Passage Stability***



Passage	MFI for EGF R (PE)
P10	16110.58
P15	14339.94
P20	14802.25

Fig6. Passage stability analysis of receptor expression by FACS. Flow cytometry surface staining of human EGF R on Human EGF R (Luc) HEK293 Reporter Cell demonstrates consistent mean fluorescent intensity across across passage10-20.

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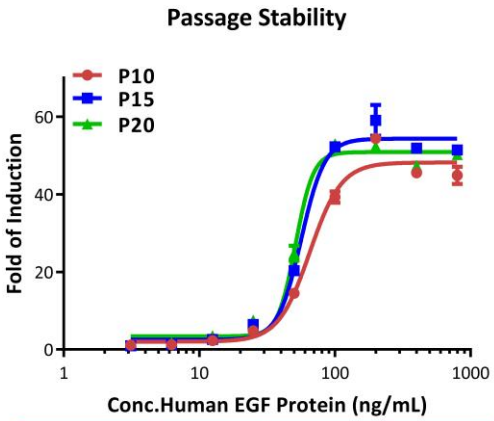

	P10	P15	P20
EC50 (ng/mL)	65.35	56.47	51.33
Max Fold	54.42	59.06	52.99

Fig7. Passage stability analysis by Signaling Bioassay. The continuously growing Human EGF R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human EGF protein. Human EGF protein stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 10-20.

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• *Related Products*

Products	**Cat.No.**
Human EGF Protein	EGF-H52H3