Human EGF R (Luc) HEK293 Reporter Cell Data Sheet

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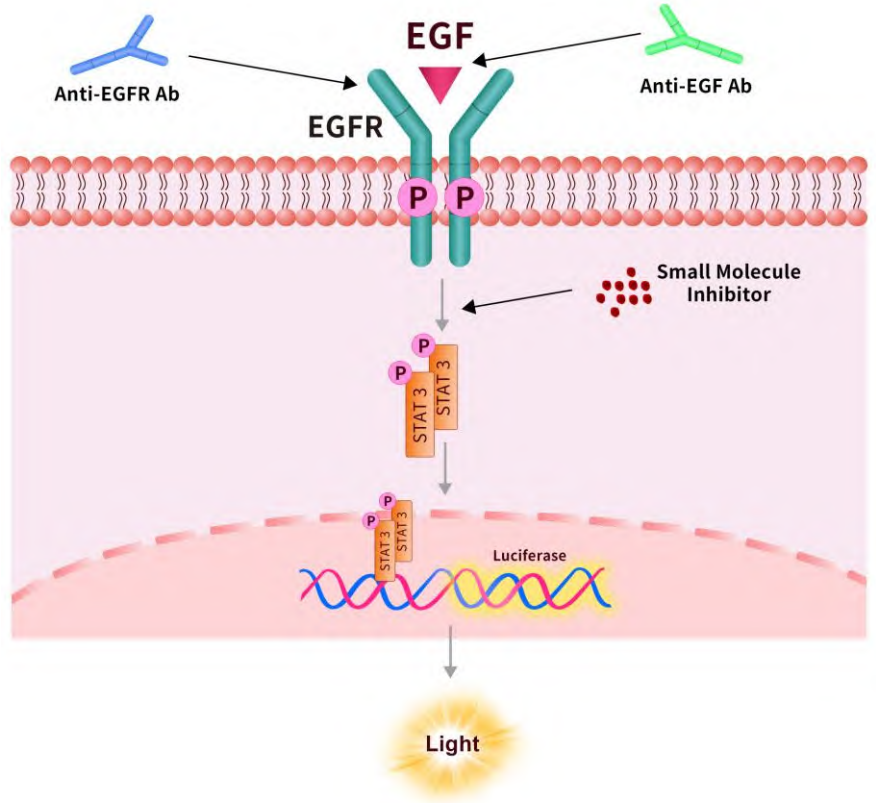
Catalog No.	Size
CHEK-ATF049	2 × (1 vial contains ~5×10^6 cells)

• *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 (Uniprot: P00533-1). 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.



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• *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 B (40 μg/mL) + 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)
- Hygromycin B (Invitrogen, Cat. No. 10687010)

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, Hygromycin B (40 μg/mL), 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 60-80% 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 60-80% confluency, proceed to the next subculture. Avoid over-confluency, as this may negatively impact cell performance in subsequent passages.

Note:

(1) 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.

(2) To ensure optimal cell health, it is essential to replace with a new T75 flask at each passage.

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• *Cryopreservation*

1. When the cell culture reaches 60-80% 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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- ***Receptor Assay***

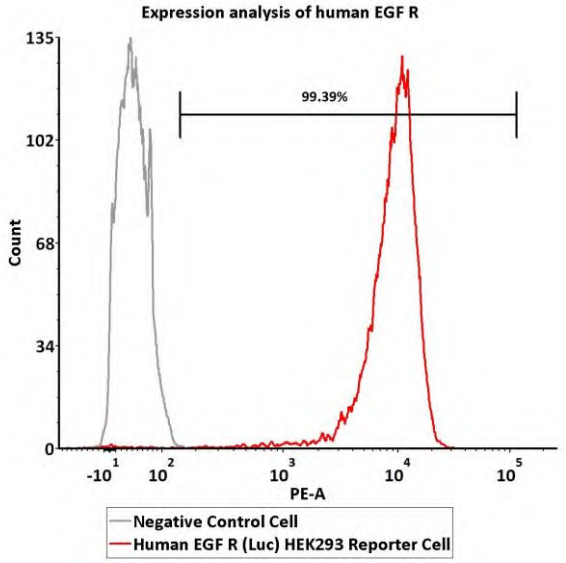

Fig1. Expression analysis of human EGF R on Human EGF R (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human EGF R (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-EGF R antibody.

- ***Signaling Bioassay***

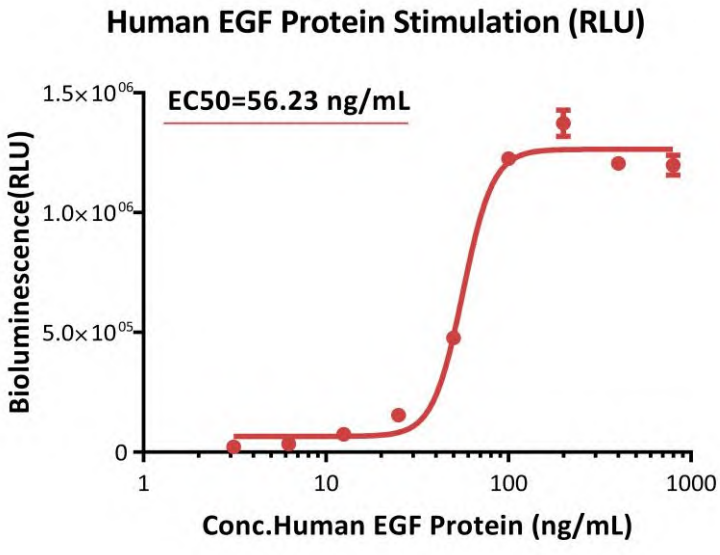

Fig2. Response to human EGF protein (RLU). The Human EGF R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human EGF protein (Cat. No. EGF-H52H3). The EC50 was approximately 56.23 ng/mL.

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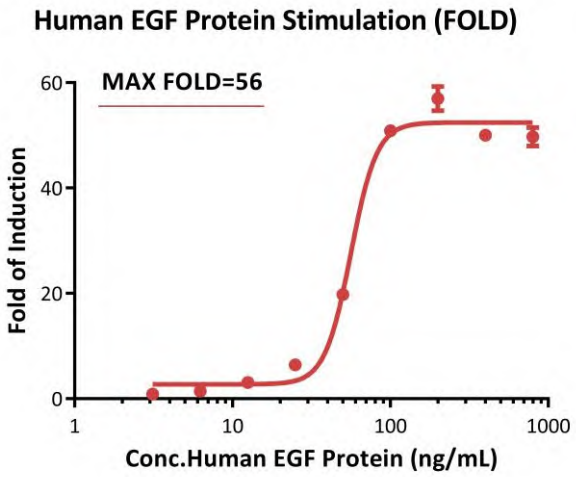

Fig3. Response to human EGF protein (FOLD). The Human EGF R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human EGF protein (Cat. No. EGF-H52H3). The max induction fold was approximately 56.

- ***Application***

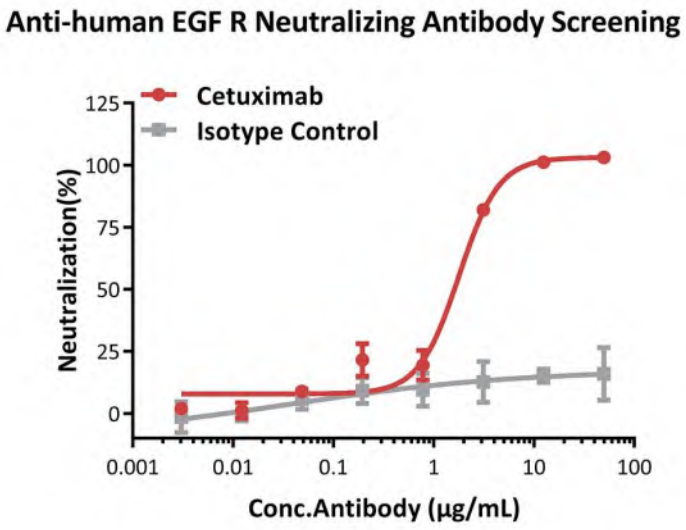

Fig4. Inhibition of human EGF protein-induced reporter activity by anti-human EGF R neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human EGF protein (Cat. No. EGF-H52H3) with a final concentration of 50 ng/mL. The EC50 of anti-human EGF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

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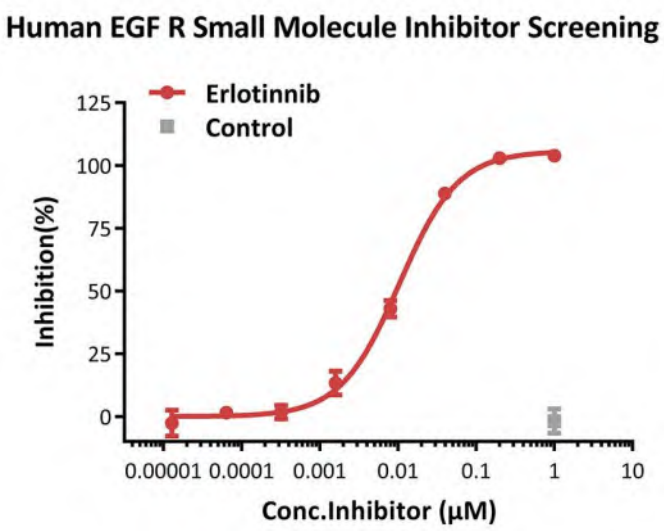

Fig5. Inhibition of human EGF protein-induced reporter activity by human EGF R small molecule inhibitor. This reporter cell was incubated with serial dilutions of inhibitors in the presence of human EGF protein (Cat. No. EGF-H52H3) with a final concentration of 50 ng/mL. The EC50 of human EGF R small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

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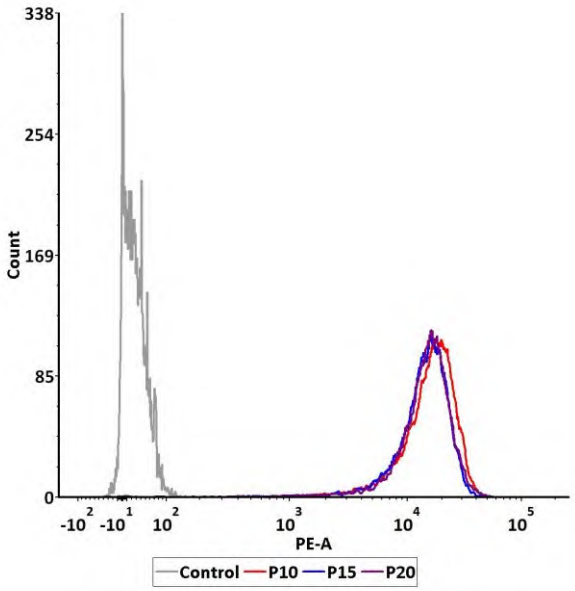
• ***Passage Stability***



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 passage 10-20.

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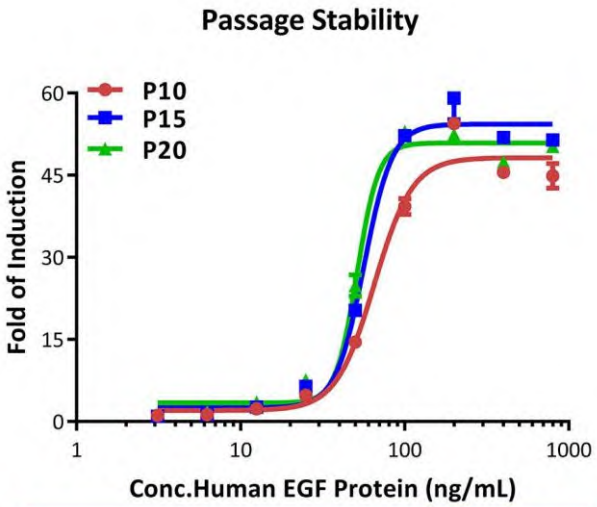

	P10	P15	P20
EC50 (ng/mL)	65.35	56.47	51.33
Max Fold	54.42	59.06	52.99

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.

• *Related Products*

Products	Cat. No.
Human EGF Protein	EGF-H52H3
Human c-MET (Luc) HEK293 Reporter Cell	CHEK-ATF144
Human TGF-beta R (Luc) HEK293 Reporter Cell	CHEK-ATF145
NFAT (Luc) Jurkat Reporter Cell	SCJUR-STF046