

Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell Data Sheet

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ACKNOWLEDGMENT

By using this Product, Customer acknowledges that he/she has read, understood, and agreed to be bound by the terms and conditions of this Limited Use and License Statement. If Customer does not agree to comply with these terms, Customer shall not open or use the Product and shall contact ACROBiosystems to arrange for return of the unused Product.

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Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell

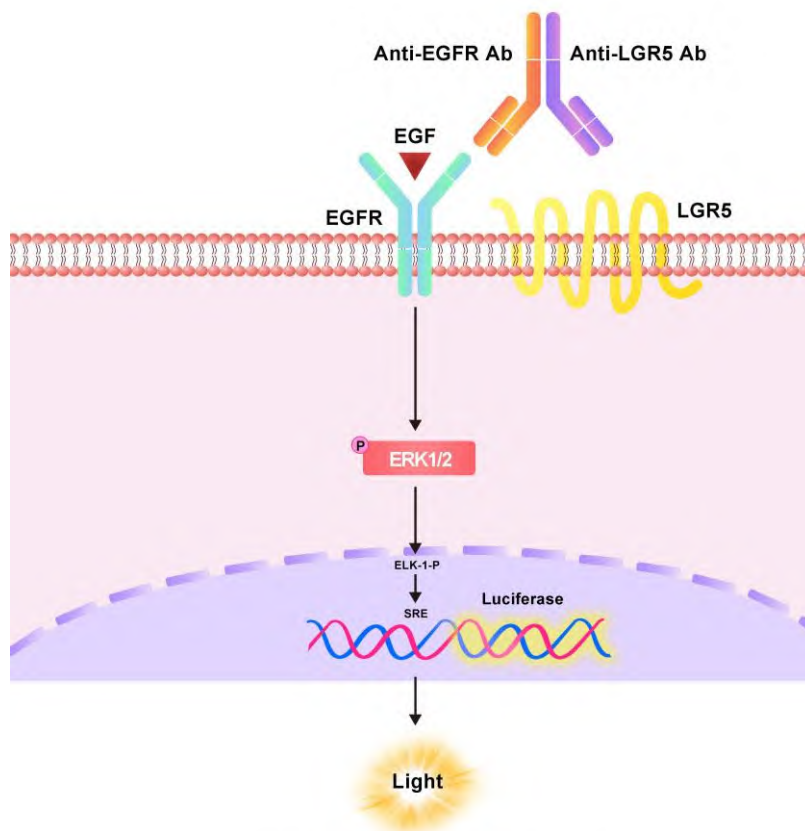
Catalog No.	Size
CHEK-ATF288	2 × (1 vial contains $\sim 5 \times 10^6$ cells)

• Description

The Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell expressing human EGFR was engineered to not only express SRE signaling response element, but also express the receptor human LGR5 (Uniprot: O75473-1). When stimulated with human EGF protein, receptor-mediated signaling can drive SRE-mediated luminescence. Neutralization of biological effect of the ligand-receptor interaction by either anti-human EGFR antibody or anti-human EGFR × LGR5 bispecific antibody results in a decrease in luminescence, with the overexpressed LGR5 exerting a further enhancement effect.

• Application

- Screen for anti-human EGFR × LGR5 bispecific antibody.



Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell

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• Cell Line Profile

Cell line	Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 µg/mL) + Hygromycin B (20 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), 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, Puromycin (2 µg/mL), Hygromycin B (20 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)
- CO2 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% CO₂ 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% CO₂ 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.

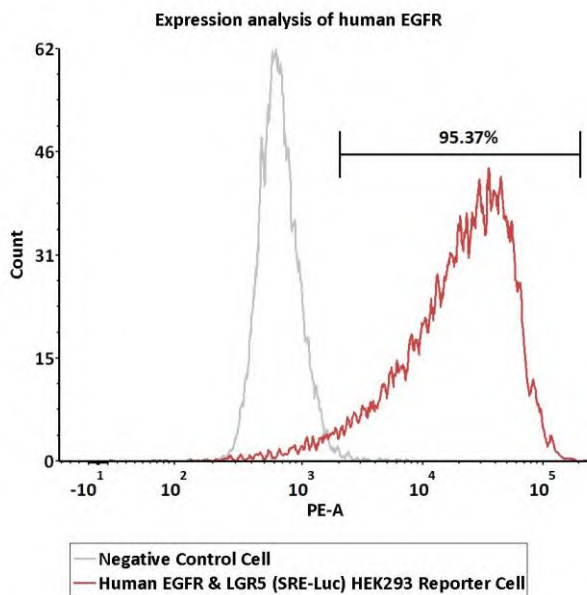
Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *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.

Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell Data Sheet

• *Receptor Assay*

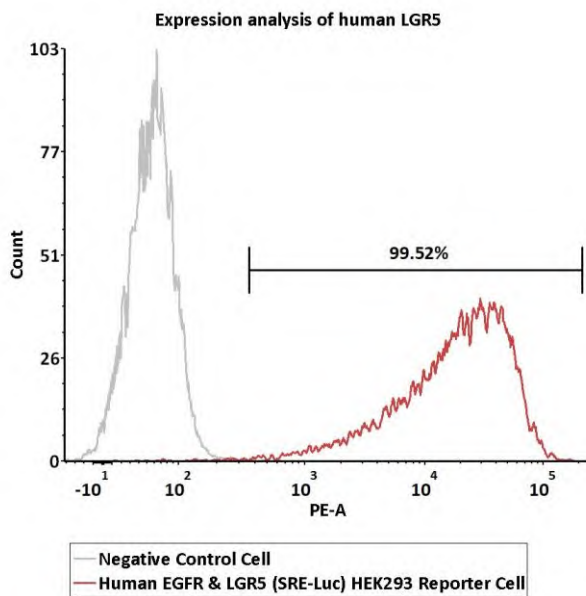


Catalog No.	Stable Cell Line	MFI for EGFR (PE)
NA	Negative Control Cell	619.22
CHEK-ATF288	Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell	21994.99

Fig1. Expression analysis of human EGFR on Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human EGFR antibody.

Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell Data Sheet

• Receptor Assay



Catalog No.	Stable Cell Line	MFI for LGR5 (PE)
NA	Negative Control Cell	55.41
CHEK-ATF288	Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell	19949.12

Fig2. Expression analysis of human LGR5 on Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human LGR5 antibody.

Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell Data Sheet

• Signaling Bioassay

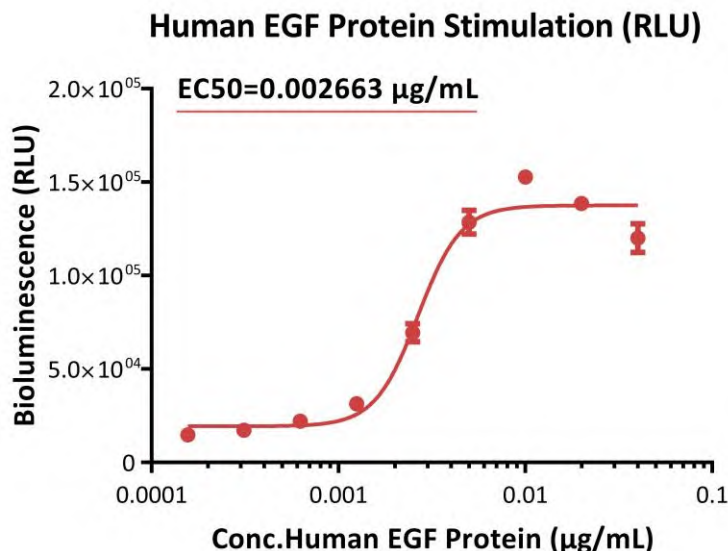


Fig3. Response to human EGF protein (RLU). The Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human EGF protein (Cat. No. EGF-H52H3). The EC50 was approximately 0.002663 µg/mL.

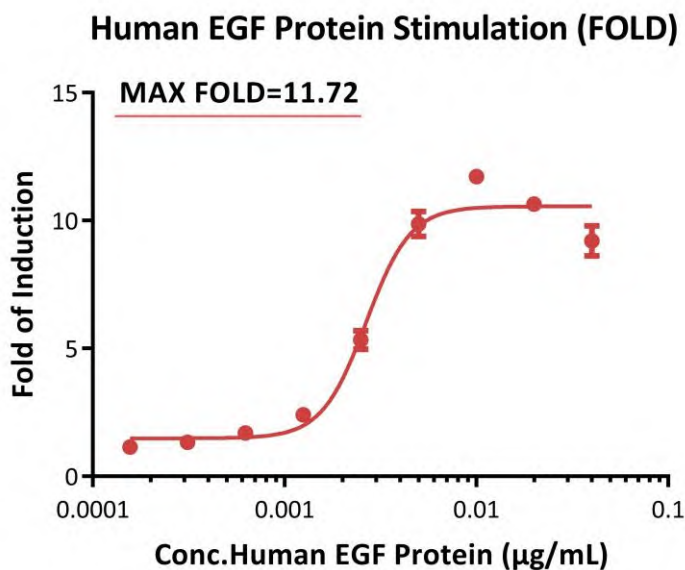


Fig4. Response to human EGF protein (FOLD). The Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell stimulated with serial dilutions of human EGF protein (Cat. No. EGF-H52H3). The max induction fold was approximately 11.72.

Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell Data Sheet

• Application

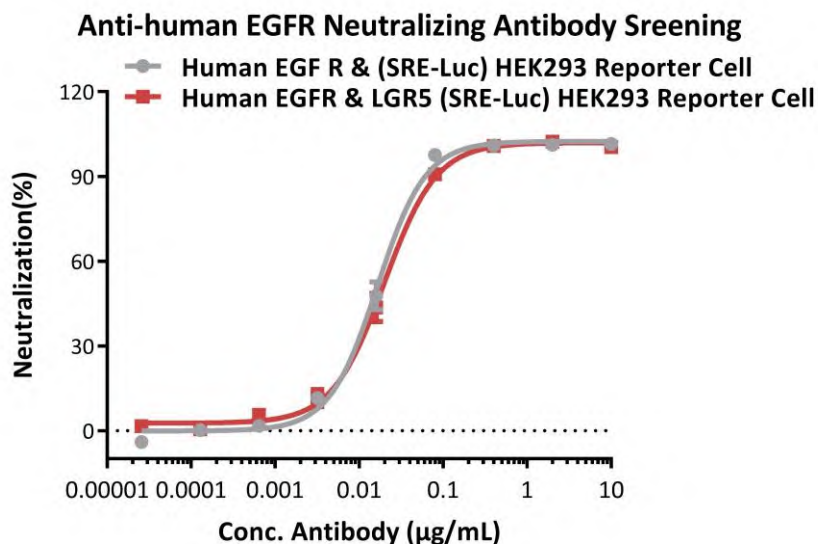


Fig5. Inhibition of human EGF protein-induced reporter activity by anti-human EGFR neutralizing antibody. The Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell and the parental Human EGF R & (SRE-Luc) HEK293 Reporter Cell (Cat. No. CHEK-ATF278) was incubated with serial dilutions of anti-human EGFR neutralizing antibodies in the presence of human EGF protein (Cat. No. EGF-H52H3) with a final concentration of 0.005 µg/mL. The EC₅₀ of anti-human EGFR neutralizing antibody determined on the Human EGF R & (SRE-Luc) HEK293 Reporter Cell was approximately 0.01652 µg/mL, and on the Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell was approximately 0.01992 µg/mL. The fold change (EC₅₀ [Human EGF R & (SRE-Luc) HEK293 Reporter Cell] / EC₅₀ [Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell]) was approximately 0.83.

Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell Data Sheet

• Application

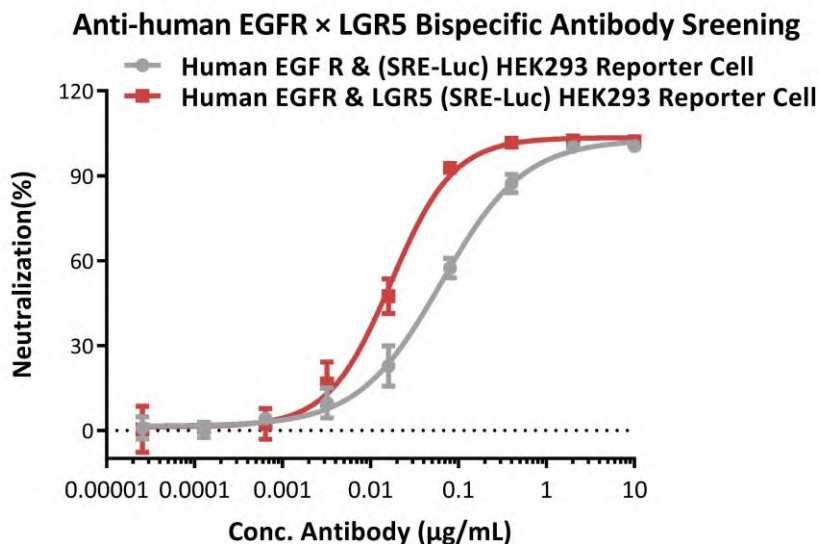


Fig6. Inhibition of human EGF protein-induced reporter activity by anti-human EGFR × LGR5 bispecific antibody. The Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell and the parental Human EGF R & (SRE-Luc) HEK293 Reporter Cell (Cat. No. CHEK-ATF278) was incubated with serial dilutions of anti-human EGFR × LGR5 bispecific antibodies in the presence of human EGF protein (Cat. No. EGF-H52H3) with a final concentration of 0.005 µg/mL. The EC₅₀ of anti-human EGFR × LGR5 bispecific antibody determined on the Human EGF R & (SRE-Luc) HEK293 Reporter Cell was approximately 0.0637 µg/mL, and on the Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell was approximately 0.01716 µg/mL. The fold change (EC₅₀ [Human EGF R & (SRE-Luc) HEK293 Reporter Cell] / EC₅₀ [Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell]) was approximately 3.71, demonstrating that the overexpressed LGR5 enhanced the neutralizing effect of the anti-human EGFR × LGR5 bispecific antibody.

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• *Related Products*

<u>Products</u>	<u>Cat.No.</u>
Human VEGF R2 (Luc) HEK293 Reporter Cell	CHEK-ATF044
NF-κB (Luc) HEK293 Reporter Cell	CHEK-ATF048
Human EGF R (Luc) HEK293 Reporter Cell	CHEK-ATF049
NFAT (Luc) HEK293 Reporter Cell	CHEK-ATF050
HEK293/Human CCR5 Stable Cell Line	CHEK-ATP043
HEK293/Human SIRP alpha Stable Cell Line	CHEK-ATP051
HEK293/Human CD20 Stable Cell Line	CHEK-ATP034
HEK293/Human ASGR1 Stable Cell Line	CHEK-ATP080
HEK293/Human TMPRSS2-HA-P2A-mGFP Stable Cell Line	CHEK-ATP101
NF-κB (Luc) Jurkat Reporter Cell	SCJUR-STF113
TCF/LEF (Luc) HEK293 Reporter Cell	CHEK-ATF114
Human NKp46 (Luc) Jurkat Reporter Cell	SCJUR-STF130
ISRE (Luc) HEK293 Reporter Cell	CHEK-ATF134
HEK293/Human CCR8 Stable Cell Line	CHEK-ATP140
Human c-MET (Luc) HEK293 Reporter Cell	CHEK-ATF144
HEK293/Human ASGR1&ASGR2 Stable Cell Line	CHEK-ATP172
Human TGF-beta R (Luc) HEK293 Reporter Cell	CHEK-ATF145
Human BMP (Luc) HEK293 Reporter Cell	CHEK-ATF188
HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line	CHEK-ATP199
HEK293/Human IDH1(132R)-P2A-mGFP&Luc Stable Cell Line	CHEK-ATP200