Human GFR alpha-1 & RET (GDNF receptor) (Luc) HEK293 Reporter Cell Data Sheet

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Human GFR alpha-1 & RET (GDNF receptor) (Luc) HEK293 Reporter Cell

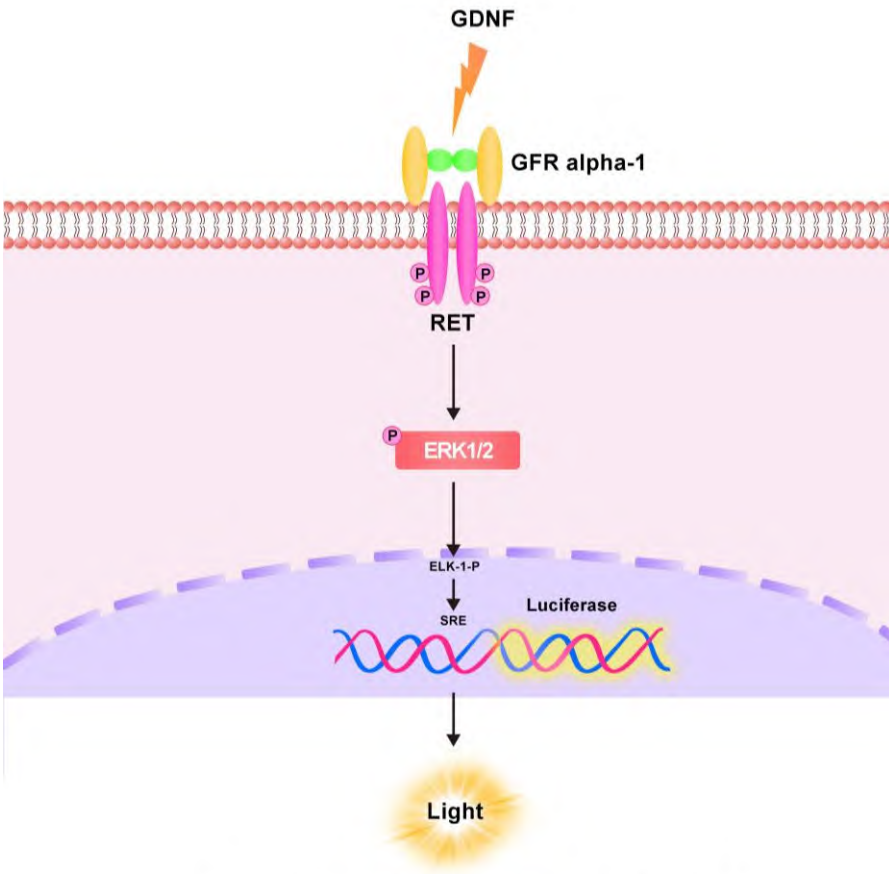
Catalog No.	Size
CHEK-ATF297	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human GFR alpha-1 & RET (GDNF receptor) (Luc) HEK293 Reporter Cell was engineered to not only express SRE signaling response element, but also express the receptors human GFR alpha-1 (Uniprot: P56159-1) and RET (Uniprot: P07949-1). When stimulated with human GDNF protein, receptor-mediated signaling can drive SRE-mediated luminescence.

• *Application*

- Bioactivity detection of human GDNF fusion protein.



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Human GFR alpha-1 & RET (GDNF receptor) (Luc) HEK293 Reporter Cell Data Sheet

• *Cell Line Profile*

Cell line	Human GFR alpha-1 & RET (GDNF receptor) (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) + Zeocin (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)
- Zeocin (Invitrogen, Cat. No. R25001)

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), Zeocin (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)
- 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:

(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.

• *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*

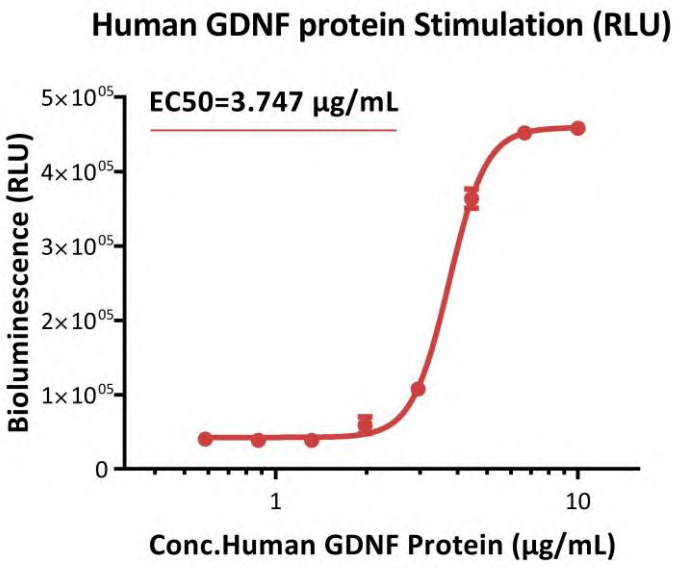

Fig1. Response to human GDNF protein (RLU). The Human GFR alpha-1 & RET (GDNF receptor) (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDNF protein (Cat. No. GDF-H5118). The EC50 was approximately 3.747 μg/mL.

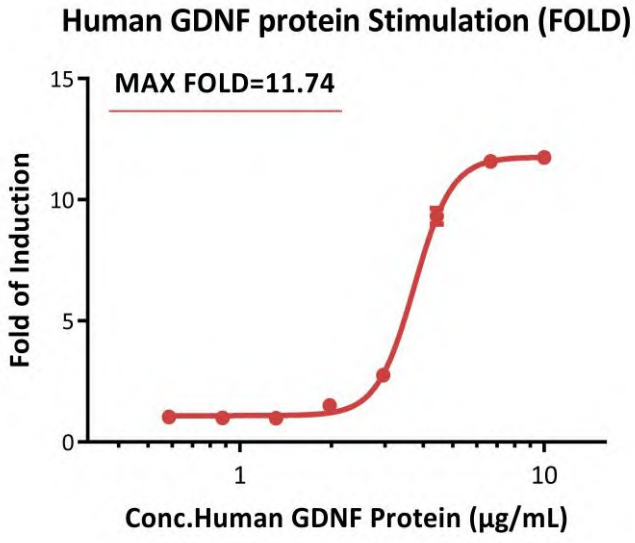

Fig2. Response to human GDNF protein (FOLD). The Human GFR alpha-1 & RET (GDNF receptor) (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human GDNF protein (Cat. No. GDF-H5118). The max induction fold was approximately 11.74.

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• *Related Products*

Products	Cat.No.
Human TrkA (Luc) HEK293 Reporter Cell	CHEK-ATF093
HEK293/Human APP (GFP) Stable Cell Line	CHEK-ATP081
HEK293/Human TrkB Stable Cell Line	CHEK-ATP082
HEK293/Human Alpha-synuclein (GFP) Stable Cell Line	CHEK-ATP085
HEK293/Human Tau-K18 (GFP) Stable Cell Line	CHEK-ATP087
Human 5-HT1A (Luc) HEK293 Reporter Cell	CHEK-ATF131
HEK293/Human SORT1 Stable Cell Line	CHEK-ATP155
HEK293/Human RAGE Stable Cell Line	CHEK-ATP156
HEK293/Human NGFR Stable Cell Line	CHEK-ATP157
HEK293/Human LDL R Stable Cell Line	CHEK-ATP158
HEK293/Human LILRB3 Stable Cell Line	CHEK-ATP159
Human CGRPR/RAMP1(Luc) HEK293 Reporter Cell	CHEK-ATF168
HEK293/Human TrkC Stable Cell Line	CHEK-ATP189
HEK293/Human TrkA Stable Cell Line	CHEK-ATP192