

### Human FGF-21 (Luc) HEK293 Reporter Cell

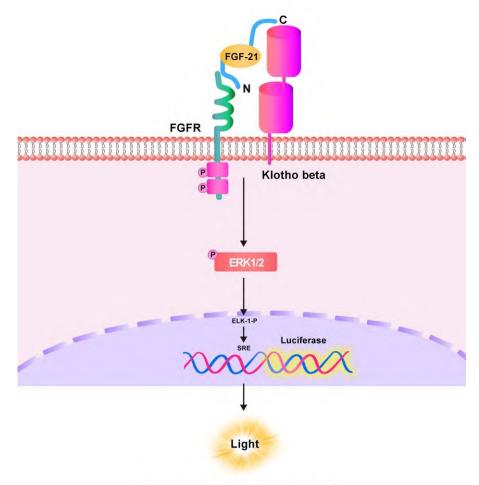
Catalog No.	Size
CHEK-ATF163	$2 \times (1 \text{ vial contains } \sim 5 \times 10^{6} \text{ cells})$

#### • Description

The Human FGF-21 (Luc) HEK293 Reporter Cell was engineered to not only express SRE signaling response element, but also express the receptor human Klotho beta (Gene ID: 152831). When stimulated with human FGF-21 protein, receptor-mediated signaling can drive SRE-mediated luminescence.

#### • Application

Bioactivity detection of human FGF-21 fusion protein.



Human FGF-21 (Luc) HEK293 Reporter Cell



### • Cell Line Profile

Cell line	Human FGF-21 (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 $\mu$ g/mL) + Hygromycin (20 $\mu$ g/mL)
Incubation	37°C with 5% CO <sub>2</sub>
Doubling Time	22-24 hours
Transduction Technique	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)
- 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
- 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)
- CO<sub>2</sub> 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% CO<sub>2</sub> 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  $^\circ\!\mathrm{C}$  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.

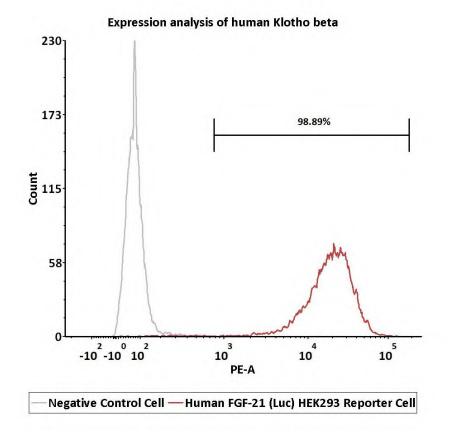


### • Cryopreservation

- 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  $5 \times 10^6$  to  $1 \times 10^7$  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

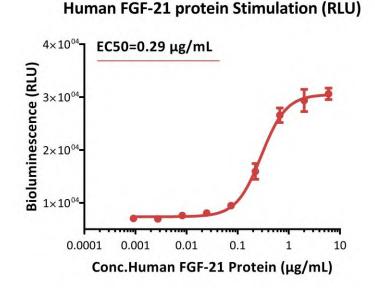


### Fig1. Expression analysis of human Klotho beta on Human FGF-21 (Luc) HEK293 Reporter Cell by FACS.

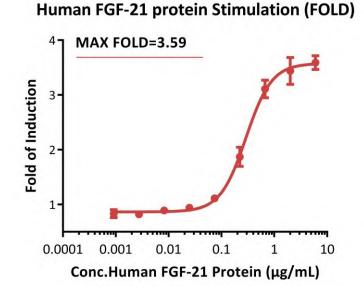
Cell surface staining was performed on Human FGF-21 (Luc) HEK293 Reporter Cell or negative control cell using anti-human Klotho beta antibody followed by staining with PE anti-human IgG antibody.



• Signaling Bioassay



**Fig2. Response to human FGF-21 protein (RLU).** The Human FGF-21 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human FGF-21 protein (Cat.No.FG1-H5243). The EC50 was approximately 0.29 µg/mL.



**Fig3. Response to human FGF-21 protein (FOLD).** The Human FGF-21 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human FGF-21 protein (Cat.No.FG1-H5243). The max induction fold was approximately 3.59.



### • Passage Stability

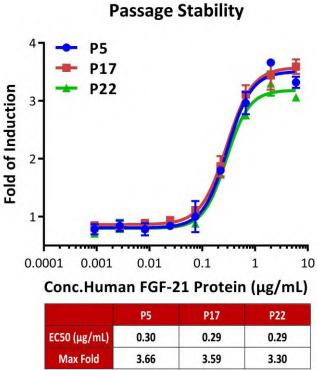


Fig4. Passage stability analysis by Signaling Bioassay. The continuously growing Human FGF-21 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human FGF-21 protein (Cat.No.FG1-H5243). Human FGF-21 protein stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 5-22.



#### • License Disclosure

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#### • Related Products

#### **Products**

Human FGF-21 Protein, His Tag

<u>Cat.No.</u> FG1-H5243