SZABO SCANDIC

Part of Europa Biosite

# Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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**Description**

The FGF-21 Responsive Luciferase Reporter HEK293 Cell Line is a HEK293 cell line engineered to express firefly luciferase under the control of the GAL4 upstream activation sequence (UAS). It overexpresses human β-Klotho (KLB, NM_175737.4) and human Elk-1 (ETS Like-1 protein) transactivation domain (Elk1-TA, amino acids 205-end) fused to the DNA binding domain (DBD) of GAL4 (GAL4 DBD, amino acid 1-147). This allows for quantitative measurement of FGF-21 (Fibroblast growth factor 21) activity through its cognate receptors and downstream signaling networks.

This cell line has been validated by stimulation with recombinant human FGF-21 and inhibition by a pan-FGFR inhibitor, Futibatinib.

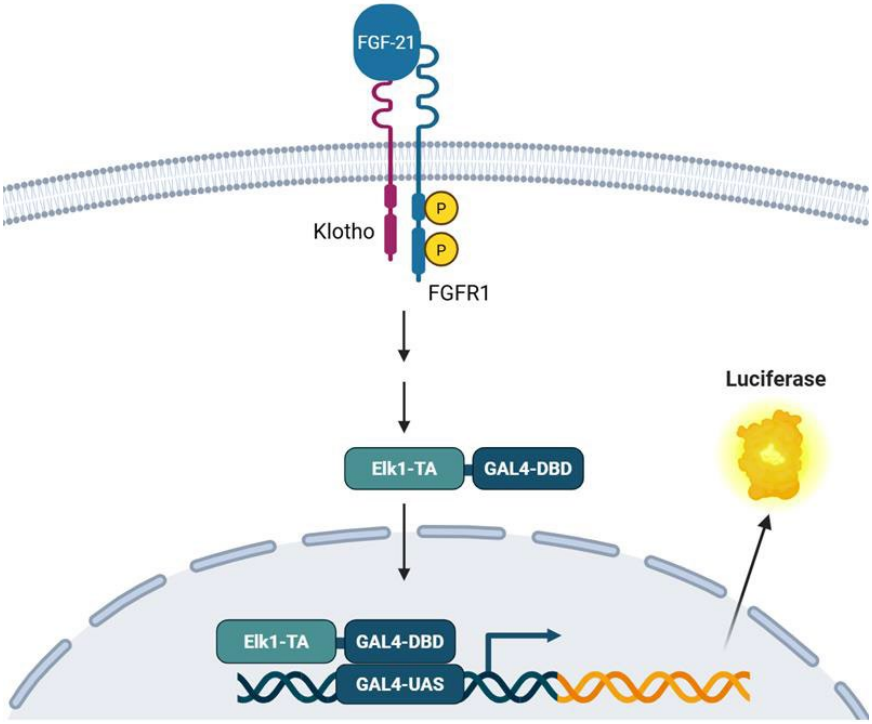


*Figure 1: Illustration of the mechanism leading to luciferase expression in response to FGF-21 treatment in FGF-21 Responsive Luciferase Reporter HEK293 Cell Line.*

**Background**

Fibroblast growth factor 21 (FGF-21) is a member of the FGF family that lacks the heparin-binding ability and can be released into circulation to act as an endocrine factor. It is found in the liver, adipose tissue, central nervous system and other tissues, with circulating FGF-21 being expressed in the liver. It is a stress-inducible hormone that plays a key role in regulating energy balance, as well as glucose and lipid homeostasis, through a heterodimeric receptor complex composed of FGF receptor 1 (FGFR1), which is a tyrosine kinase, and co-receptor β-Klotho. In the liver, the effects of transcription factor PPARα (Peroxisome proliferator-activated receptor alpha) are mediated by increased production of FGF-21. FGF-21 regulates sugar intake and insulin sensitivity. Mutations in FGF-21 have been linked to obesity and type-2 diabetes (T2D) risk, therefore FGF-21 has attracted attention as a therapeutic target for those disorders. Further studies are required to elucidate the detailed mechanisms of action of FGF-21 and the benefits of drugs targeting this hepatokine.





BPS Bioscience

● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

**Application(s)**
Screen or characterize FGF-21 analogs or antagonists.

**Materials Provided**

| Components | Format |
|---|---|
| 2 vials of frozen cells | Each vial contains ≥1 x $10^6$ cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796) |

**Parental Cell Line**
HEK293, Human Embryonic Kidney, epithelial-like cells, adherent.

**Mycoplasma Testing**
The cell line has been screened to confirm the absence of Mycoplasma species.

**Materials Required but Not Supplied**

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

*Materials Required for Cell Culture*

| Name | Ordering Information |
|---|---|
| Thaw Medium 1 | BPS Bioscience #60187 |
| Growth Medium 1Y | BPS Bioscience #82535 |

*Materials Required for Cellular Assay*

| Name | Ordering Information |
|---|---|
| Assay Medium 1A | BPS Bioscience #79805 |
| Recombinant Human FGF-21 | R&D Systems #2539-FG |
| Recombinant Human FGF-19 | R&D Systems #969-FG |
| Futibatinib | MCE #HY-100818 |
| ONE-Step™ Luciferase Assay System | BPS Bioscience #60690 |
| 96-well tissue culture treated white clear-bottom assay plate | Corning #3610 |
| Luminometer | |

**Storage Conditions**

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

**Media Formulations**
For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Note that using similar but not BPS Bioscience validated reagents can result in suboptimal performance.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.
Cells should be grown at 37℃ with 5% $CO_2$. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

*Media Required for Cell Culture*

*Thaw Medium 1 (BPS Bioscience #60187):*
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, and 1% Penicillin/Streptomycin.

*Growth Medium 1Y (BPS Bioscience #82535):*
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 400 µg/ml of G418, 100 µg/ml Hygromycin, and 0.5 µg/ml of Puromycin.

*Media Required for Functional Cellular Assay*

*Assay Medium 1A (BPS Bioscience #79805):*
Opti-MEM medium supplemented with 1% Penicillin/Streptomycin.

**Cell Culture Protocol**
**Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.**

*Cell Thawing*

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 15 ml conical tube.

   **Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.**

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 1 to the conical tube containing the cells. Thaw Medium 1 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% $CO_2$ incubator.

6. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% $CO_2$ incubator at 37°C until the cells are ready to passage.

   *Note: Recovery of the frozen cells can take longer than a week. Change medium to fresh Thaw Medium 1 after a week.*

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1Y.

*Cell Passage*

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without $Ca^{2+}$/$Mg^{2+}$, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1Y and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1Y.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 every week.

*Cell Freezing*

1. Aspirate the medium, wash the cells with PBS without $Ca^{2+}$/$Mg^{2+}$ and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1Y and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at 1~2 x $10^6$ cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

**Validation Data**

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- Assay A should include “Stimulated Cells”, “Background Control” and “Unstimulated Control” conditions.
- Assay B should include “Stimulated Cells, No Compound”, “Stimulated Cells, Test Compound”, “Background Control” and “Unstimulated Control” conditions.

**A. Dose Response of FGF-21 Responsive Luciferase Reporter HEK293 Cell Line to recombinant FGF-21 protein (96-well)**

1. Seed cells at a density of 30,000 ~ 40,000 cells per well in 90 µl of Assay Medium 1A into a white clear-bottom 96-well microplate. Leave empty wells as cell-free control wells (“Background Control”).

2. Prepare a serial dilution of recombinant FGF-21 protein in Assay Medium 1A at 10x the final test concentrations (10 µl/well).

3. Add 10 µl of diluted FGF-21 to the "Stimulated Cells" wells.

4. Add 10 µl of Assay Medium 1A to the "Unstimulated Control" wells.

5. Add 100 µl of Assay Medium 1A to "Background Control" wells (cell-free wells).

6. Incubate at 37°C with 5% $CO_2$ for 20 ~ 24 hours.

7. Add 100 µl of ONE-Step™ Luciferase reagent per well.

8. Incubate at Room Temperature (RT) for ~10 minutes.

9. Measure luminescence using a luminometer.

10. The "Background Control" luminescence value should be subtracted from all readings.

11. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

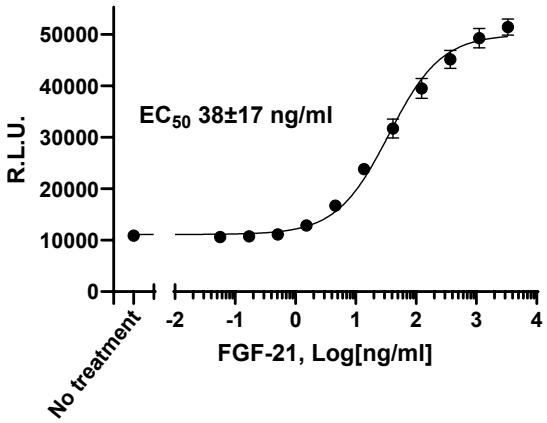


*Figure 2. Dose response curve of FGF-21 Responsive Luciferase Reporter HEK293 Cell Line to recombinant FGF-21 protein (96-well format).*
FGF-21 Luciferase Reporter HEK293 cells were incubated with increasing concentrations of FGF-21 for 24 hours. Luciferase activity was measured using ONE-Step™ Assay System (#60690).

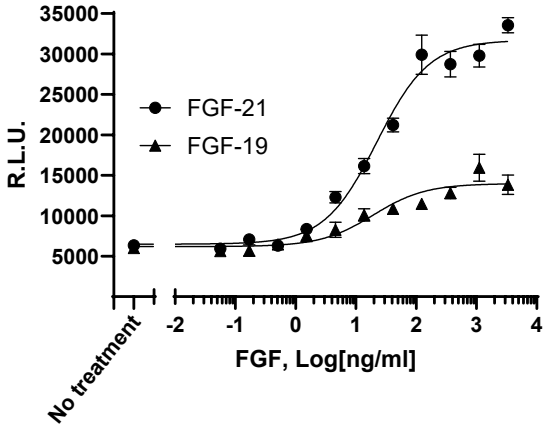


*Figure 3. Dose response of FGF-21 Responsive Luciferase Reporter HEK293 Cell Line to recombinant FGF-21 and FGF-19 proteins (96-well format).*
FGF-21 Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of FGF-21 and FGF-19. Luciferase activity was measured using ONE-Step™ Assay System (#60690).

**B. Inhibition of FGF-21 induced reporter activity by Futibatinib in FGF-21 Responsive Luciferase Reporter HEK293 Cell Line**

1. Seed cells at a density of 30,000 - 40,000 cells per well in 80 µl of Assay Medium 1A into a white clear-bottom 96-well microplate. Leave empty wells as cell-free control wells ("Background Control").

2. Prepare a serial dilution of Futibatinib in Assay Medium 1A at 10x final testing concentrations (10 µl/well). For example, to test 100 nM Futibatinib, prepare 100 µM Futibatinib in DMSO and dilute it 100-fold with Assay Medium 1A. This results in 1 µM Futibatinib in Assay Medium 1A containing 1% DMSO. This is the diluted Futibatinib at 10x final test concentration.

3. Add 10 µl of diluted Futibatinib to the "Stimulated Cells, Test Compound" wells.

4. Add 10 µl of Assay Medium 1A containing 1% DMSO to the "Stimulated Cells, No Compound" and "Unstimulated Control" (100% activity and 0% activity respectively).

5. Incubate at 37°C with 5% $CO_2$ for 1 hour.

6. Prepare FGF-21 in Assay Medium 1A at 10x $EC_{90}$ concentration, i.e. at 3 µM (10 µl/well).

7. Add 10 µl of diluted FGF-21 to the "Stimulated Cells, Test Compound" and "Stimulated Cells, No Compound" wells.

8. Add 10 µl of Assay Medium 1A to the "Unstimulated" wells.

9. Add 100 µl of Assay Medium 1A to the "Background Control" wells.

10. Incubate at 37°C with 5% $CO_2$ for 20 to 24 hours.

11. Add 100 µl of ONE-Step™ Luciferase reagent per well.

12. Incubate RT for ~10 minutes.

13. Measure luminescence using a luminometer.

14. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The percent luminescence is the average background control-subtracted luminescence of the inhibitor treated wells divided by the average background control-subtracted luminescence of the "Stimulated Cells, No Compound" wells (FGF-21 added but no Futibatinib) multiplied by 100:

$$\%\ Luminescence = \left(\frac{Luminescence\ of\ \ inhibitor\ wells - avg.background}{Avg.Luminescence\ of\ Upositive\ control\ wells - avg.background}\right) X\ 100$$

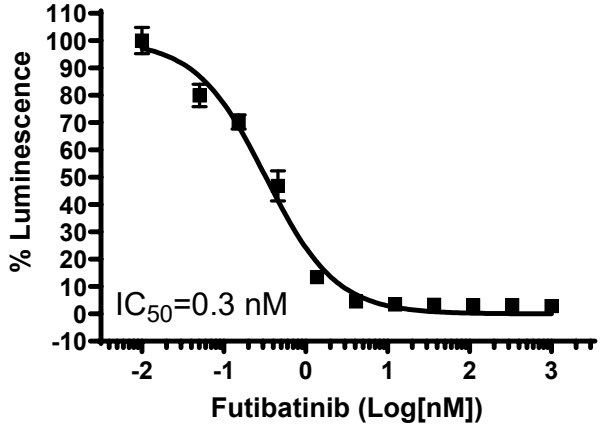


*Figure 4. Inhibition of FGF-21 induced reporter activity by Futibatinib in FGF-21 Responsive Luciferase Reporter HEK293 Cell Line.*
FGF-21 Luciferase Reporter HEK293 cells were treated with increasing concentrations of a pan-FGFR inhibitor, Futibatinib, and incubated for 1 hour before stimulation with recombinant FGF-21 protein, followed by incubation for 20-24 hours in a $CO_2$ incubator. Luciferase activity was measured with ONE-Step™ Luciferase Assay System.

*Data shown is representative.*

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**Troubleshooting Guide**
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

**References**

Geng L., *et al*., 2020 *Nature Review Endocrinology* 16: 654-667.
Lin X., *et al.*, 2017 *Biomedical Reports* 6(5):495-502.
Yie J., *et. al.*, 2008 *FEBS Letter* 583 (1): 19-24.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| PPARα (Peroxisome proliferator-activated receptor alpha)-GAL4 Luciferase Reporter HEK293 Cell Line | 82837 | 2 vials |
| TRβ-GAL4 Luciferase Reporter HEK293 Cell Line | 82175 | 2 vials |
| TRα-GAL4 Luciferase Reporter HEK293 Cell Line | 82633 | 2 vials |
| GAL4 Luciferase Reporter HEK293 Cell Line | 60656 | 2 vials |
| GAL4 DBR-GR Lentivirus | 78632 | 500 µl x 2 |
| GR-GAL Luciferase Reporter Jurkat Cell Line | 78632 | 2 vials |

*Version 090525*