Determination of neutralizing antibodies against FGF21 using *iLite*® FGF21 Assay Ready Cells

For research and professional use only. Not for use in diagnostic procedures.

This application note contains a suggested protocol and performance data.
Each individual laboratory must set up their own method and perform relevant validations.

Background

Human fibroblast growth factor 21 (FGF21) is a member of the atypical fibroblast growth factor family including FGF19 and FGF23 in human. FGF21 lacks the heparin-binding domain of conventional FGFs and can consequently diffuse throughout the body, and function as a hormone. FGF21 stimulates glucose uptake in adipocytes which is additive with insulin (1). Prolonged therapy with biological drugs such as FGF21 can lead to development of neutralizing antibodies, which could inhibit the effect of FGF21.

Principle of the assay

The *iLite*® FGF21 Assay Ready Cells are engineered cells optimized to express Firefly luciferase under the control of an FGF21 responsive promoter. When FGF21 binds to the hetero-dimeric cell surface receptor, composed of the tyrosine kinase FGFR1c receptor and b-Klotho, it activates the FGF21 regulated Firefly luciferase reporter gene construct. After addition and incubation with a luciferase substrate the Firefly luciferase signal can be measured in a luminometer. The luminescence signal is proportional to the amount of functionally active FGF21 in the sample. In the presence of neutralizing antibodies against FGF21, the amount of active FGF21 is reduced, resulting in a decreased Firefly luciferase production and subsequently lower luminescence signal. The Firefly luciferase signal is inversely proportional to the number of neutralizing antibodies in a sample. The *iLite*® FGF21 Assay Ready Cells can therefore be utilized as a highly sensitive assay for determination of anti-FGF21 neutralizing antibodies in test samples including human serum.

Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite® FGF21 Assay Ready Cells	Svar Life Science	BM3071
Diluent (DMEM containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin).	Gibco	31966-021 (DMEM) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
Anti-FGF21 antibody	Abcam	Ab64857
FGF21 or analogues	R&D	2539-FG
Firefly/Renilla luciferase substrate	Promega	E2920, Dual-Glo Luciferase Assay System
Plate; White walled micro well plate suitable for luminescence	PerkinElmer	6005680
Microplate Luminometer with appropriate reading software – no filter on luminometer	Contact Svar Life Science for list of recommended suppliers	NA
Incubator, 37 °C with 5% CO_2	NA	NA
Water bath, 37 °C	NA	NA
Single-channel and multi-channel pipettes with polypropylene disposable tips	NA	NA
Polypropylene tubes or plate for dilution	NA	NA

Single-use polypropylene reservoir	NA	NA
Plate shaker	NA	NA
Timer	NA	NA

Protocol

Preparation of anti-FGF21 neutralizing antibody

Anti-FGF21 antibody from Abcam has successfully been used to neutralize FGF21 and inhibit the FGF21 regulated Firefly luciferase expression in *iLite*® FGF21 Assay Ready Cells (refer to the table and graph below).

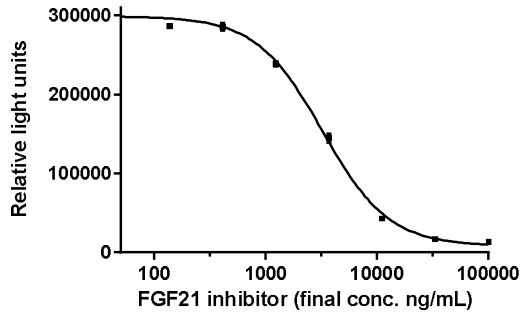

Figure 1. Example of a calibration curve of FGF21 neutralizing antibodies.

Final FGF21 conc. 50 ng/mL	Anti-FGF21 antibody Suggested calibrator solution concentrations, ng/mL
A	400 000
B	133 333
C	44 444
D	14 815
E	4 938
F	1 646
G	549
H	0

Table 1. Suggested solution concentrations of FGF21 neutralizing antibody.

Assay preparation and incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicate.
2. Perform a serial dilution of the reference anti-FGF21 antibody. Ensure matrix consistency between reference antibody solutions, control solutions, and sample solutions.
3. Add 20 µL of the reference anti-FGF21 antibody dilutions, controls and samples to assigned wells (final concentration will be a quarter of solution concentration).
4. Add 20 µL of 200 ng/mL FGF21 to all wells (final concentration will be 50 ng/mL FGF21).
5. Place the lid on the plate, mix and incubate the plate for 30 minutes at 37°C with 5% CO_2.
6. Thaw a vial of *iLite*® FGF21 Assay Ready Cells in a 37°C water bath with gentle agitation. The cell suspension is mixed very carefully ten times with a pipette to ensure a homogeneous distribution of cells.
7. Dilute 250 µL of cell suspension with 5.75 mL of Diluent
8. Add 40 µL of diluted cells to each well.
9. Place the lid on the plate, mix and incubate for 6 hours at 37 °C with 5% CO_2.

Adding substrate solutions

10. Equilibrate the plate and the substrate solutions to room temperature.
11. Prepare the **Firefly luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix and protect the plate from light. After 10 minutes incubation at room temperature read the plate in a luminometer.
12. If appropriate, prepare the **Renilla luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix and protect the plate from light. After 10 minutes incubation at room temperature read the plate in a luminometer.

Normalization readout

The reporter gene used for result normalization, Renilla luciferase, is under the control of a tyrosine kinase promoter, and thus constitutively expressed. Unspecific effects such as serum matrix effects or differences in cell number can be obviated by relating the specific Firefly signal with the Renilla normalization signal through simple division.

In the case of the growth factor FGF21, high concentrations can result in a quantifiable effect on the general machinery of the cell, such as the transcription rate of polymerases or the activity of certain elongation factors. This highly reproducible effect is seen as an increase in the normalization gene readout, proportional to the increase of FGF21 concentration. In the case of the neutralizing assay, the normalization gene readout will decrease in proportion to the increase in neutralizing antibodies, as the amount of free FGF21 is reduced (see Figure 2 below). Normalization against the Renilla signal will compensate for non-specific effects such as serum matrix effects or differences in cell number, while also excluding the effects of FGF21 on the cellular machinery in general, the result can be seen below.

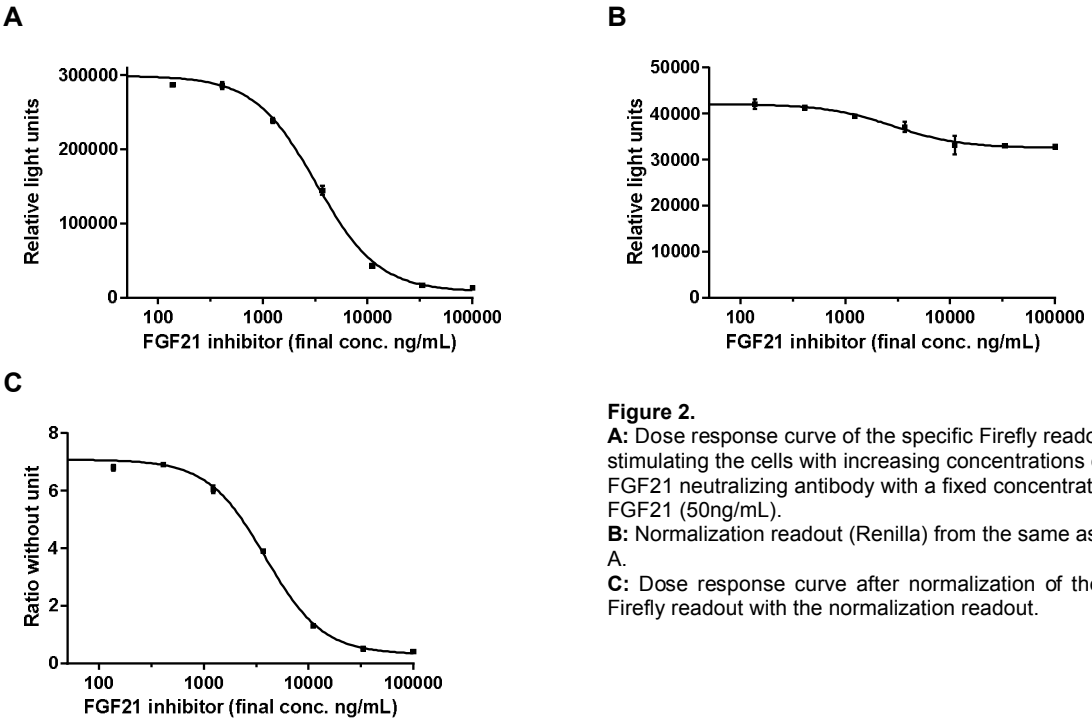

Figure 2.
A: Dose response curve of the specific Firefly readout, when stimulating the cells with increasing concentrations of anti-FGF21 neutralizing antibody with a fixed concentration of FGF21 (50ng/mL).
B: Normalization readout (Renilla) from the same assay as in A.
C: Dose response curve after normalization of the specific Firefly readout with the normalization readout.

Precautions

- This application note is intended for professional laboratory research use only. The data and results originating from following the Application Note should not be used either in diagnostic procedures or in human therapeutic applications.
- Use and handle the material and instruments referenced according to the suppliers'/manufacturers' instructions or product specifications accompanying the individual material and instruments.
- Dispose of all sample specimens, infected or potentially infected material in accordance with good microbiological practice. All such materials should be handled and disposed as though potentially infectious.
- Residues of chemicals and preparations are generally considered as biohazardous waste and should be inactivated prior to disposal by autoclaving or using bleach. All such materials should be disposed in accordance with established safety procedures.

Proprietary Information

In accepting delivery of *iLite*® Assay Ready Cells the recipient agrees not to sub-culture these cells, attempt to sub-culture them or to give them to a third-party recipient, and only to use them directly in assays. *iLite*® cell-based products are covered by patents which are the property of Svar Life Science AB and any attempt to reproduce the delivered *iLite*® Assay Ready Cells is an infringement of these patents.

QUICK GUIDE

Determination of neutralizing antibodies against FGF21 using *iLite*® FGF21 Assay Ready Cells

1 Sample dilution

- Equilibrate reagents and samples to room temperature – **do not thaw cells and substrate reagents at this stage**
- Serial dilute reference anti-FGF21 antibody
- Add 20 µL of reference anti-FGF21 antibody solutions, controls and samples to pre-assigned wells
- Add 20 µL of FGF21 to each well

2 Incubation 30 min

- Incubate at 37 °C with 5% CO_2 for 30 minutes

3 Add cells

- Thaw the vial of cells in a 37°C water bath. Mix cell suspension with a pipette in order to ensure a uniform cell suspension. Dilute the cells
- Add 40 µL of diluted cells to each well

4 Incubation 6 h

- Incubate at 37°C with 5% CO_2 for 6 hours

5 Read plate

- Equilibrate the plate to room temperature
- Prepare the **Firefly luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix. Protect the plate from light. After 10 min incubation read in a luminometer
- If appropriate, prepare the **Renilla luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix. Protect the plate from light. After 10 min incubation read in a luminometer

Troubleshooting and FAQ

Please consult the Svar Life Science website www.svarlifescience.com

References

1. **Kharitonenkov A, et.al.** (Jun 2005). "*FGF-21 as a novel metabolic regulator*". The Journal of Clinical Investigation 115 (6): 1627–35.