

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

*This application note contains a suggested protocol and performance data.
Each individual laboratory must set up their own method and perform relevant validations.
For research and professional use only.*

Background

Human fibroblast growth factor 21 (FGF21) is a member of a family of the atypical fibroblast growth factors that include FGF19 and FGF23 in man. 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 and the effect on glucose uptake is additive with insulin (1). Prolonged therapy with biological drugs such as FGF21 may lead to development of neutralizing antibodies, which may counteract 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. The Firefly luciferase signal can be measured in a luminometer following addition and incubation with a luciferase substrate. The Firefly luciferase signal is proportional to the functional activity of FGF21 in the sample. In the presence of neutralizing antibodies against FGF21, the amount of free FGF21 is reduced, resulting in a decreased Firefly luciferase production. The Firefly luciferase signal is inversely proportional to the amount 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 |
|---|--|--|
| <i>iLite</i> [®] FGF21 Assay Ready Cells | Euro Diagnostica | BM3071 |
| Diluent (DMEM containing 9% FBS and 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 Euro Diagnostica 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).

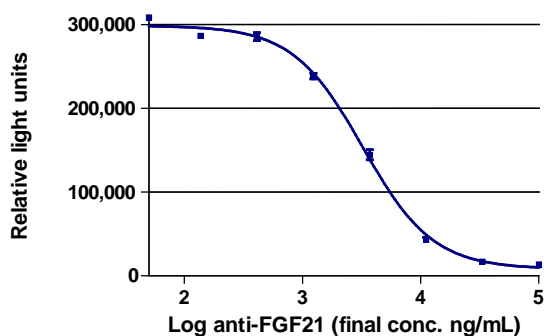


Figure 1. Example of a calibration curve of FGF21 neutralizing antibodies.

| Final FGF21 conc. 50 ng/mL | Anti-FGF21 antibody |
|-------------------------------|--|
| | Suggested 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.

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 μ L of the reference anti-FGF21 antibody dilutions, controls and samples to assigned wells.
4. Add 20 μ L of 200 ng/mL FGF21 to all wells.
5. Place the lid on the plate, mix and incubate the plate for 30 minutes at 37°C with 5% CO₂.
6. Thaw the 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 in order to ensure a uniform solution of cells.
7. Dilute 250 μ L of cells with 5.75 mL of Diluent
8. Add 40 μ L of diluted cells to each well.
9. Place the lid on the plate, mix and incubate for 6 hours at 37 °C with 5% CO₂.

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 μ L per well. Mix and protect the plate from light. Read in a luminometer after 10 minutes incubation at room temperature.
12. If appropriate, prepare the **Renilla luciferase** substrate according to the supplier's instructions and add 80 μ L per well. Mix and protect the plate from light. Read in a luminometer after 20 minutes incubation at room temperature.

Normalization readout

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

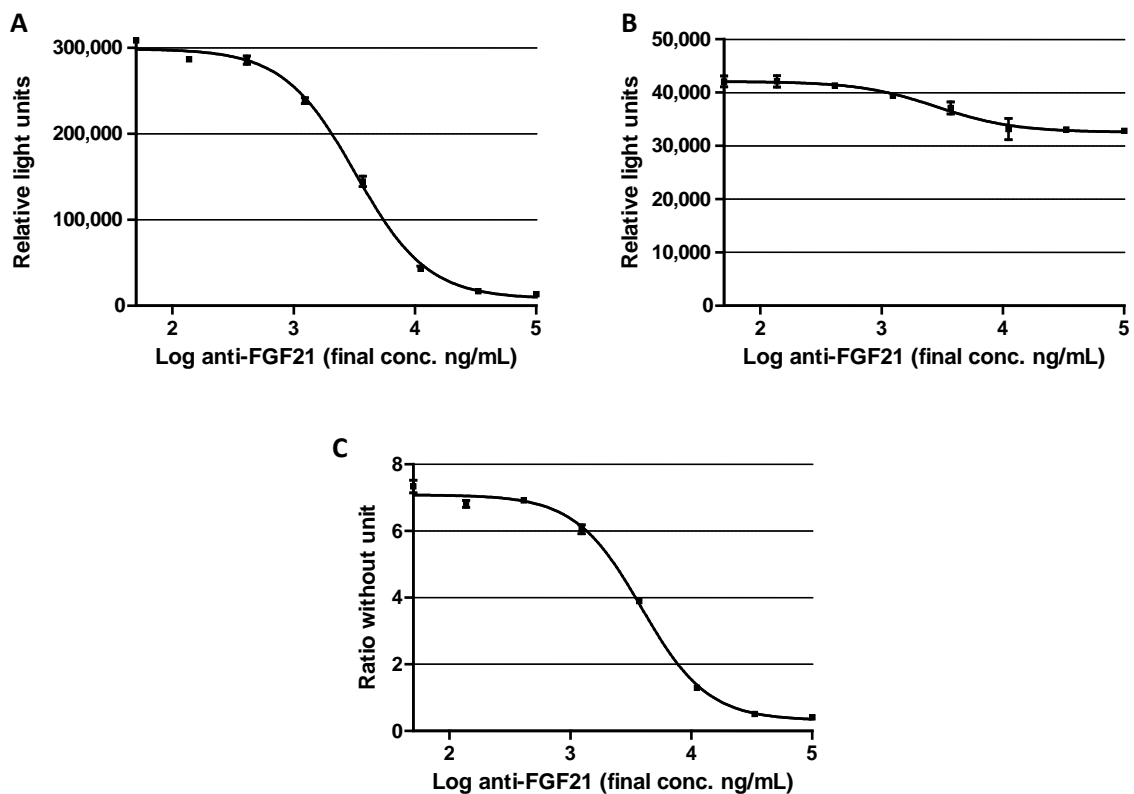


Figure 2.

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.

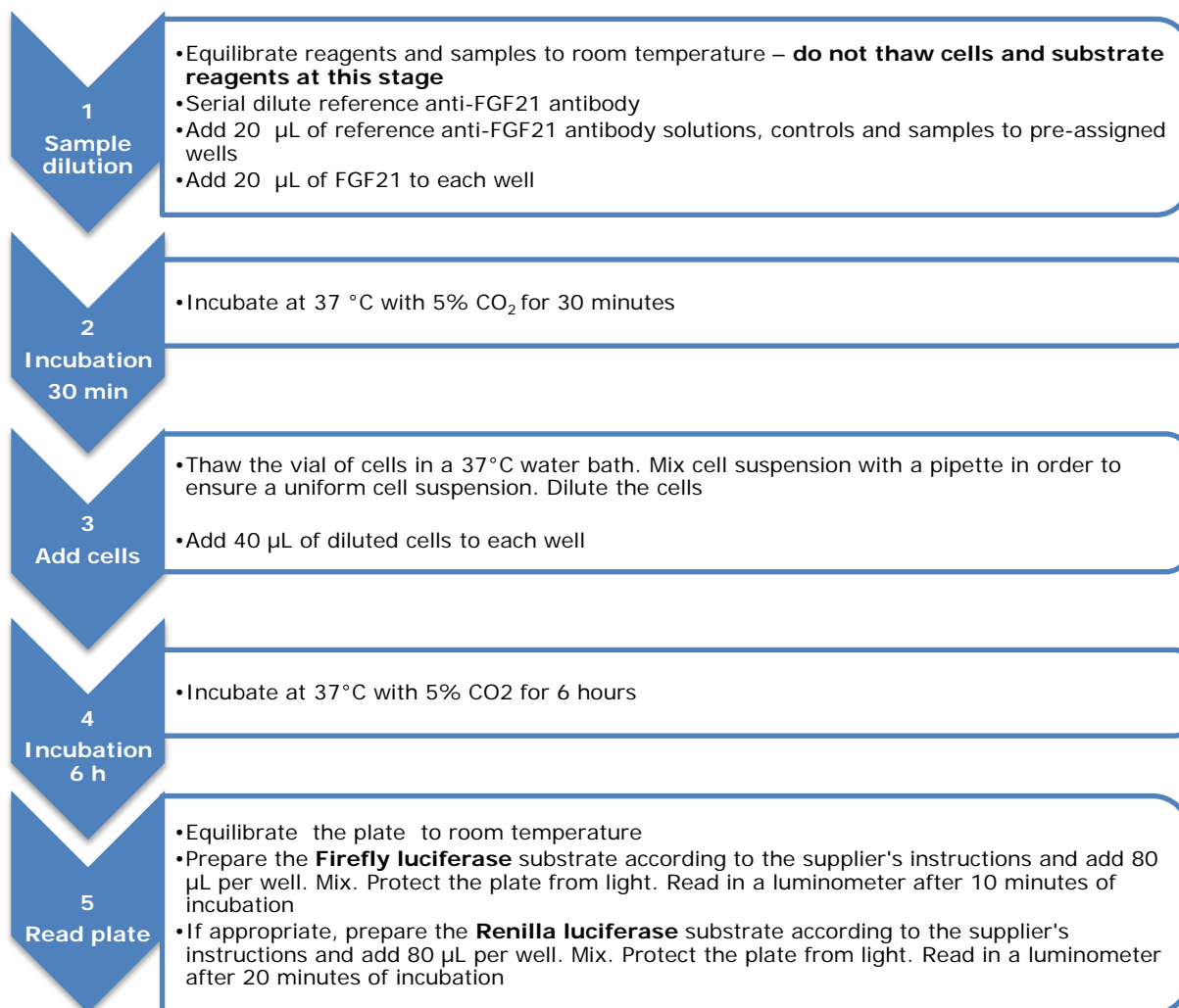
Precautions

- 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 of in accordance with established safety procedures.

Propriety 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 Euro Diagnostica 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



Troubleshooting and FAQ

Please consult Euro Diagnostica's website www.eurodiagnostica.com

References

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