Quantification of 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 a family of the atypical fibroblast growth factors that include 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 and the effects on glucose uptake is additive with insulin (1).

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. Binding of FGF21 to the hetero-dimeric cell surface receptor composed of the tyrosine kinase FGFR1c receptor and β-Klotho results in activation of 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 (Fig.1).

Specimen collection

The *iLite*® FGF21 Assay Ready Cells can be used for measuring the functional activity of FGF21 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% FBS and 1% Penicillin-Streptomycin)	Gibco	31966-021 (DMEM) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
FGF21 or analogues	R&D Systems Inc.	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

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Protocol

Preparation of calibrators (FGF21)

FGF21 from R&D Systems Inc. has successfully been used to stimulate the *iLite*® FGF21 Assay Ready Cells. The below table shows the dilutions of FGF21, used for QC release of the *iLite*® FGF21 Assay Ready Cells.

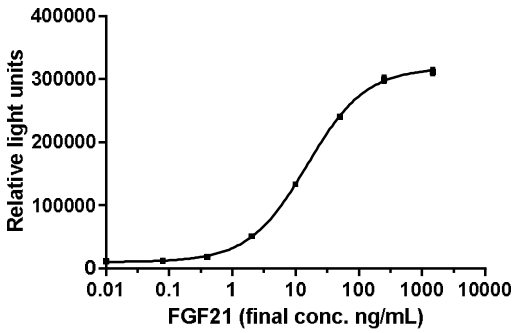

Figure 1. Example of FGF21 calibration curve

Calibrator	FGF21
	Suggested calibrator solution conc. (ng/ml)
A	3000
B	500
C	100
D	20
E	4.0
F	0.80
G	0.16
H	0

Table 1 Suggested calibrator solution concentrations for FGF21

Assay preparation and incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicates.
2. Dilute calibrators, controls and samples to fall within the expected **in-assay concentration range** of 0-1500 ng/mL.
3. Add 40 µL calibrators, controls and samples in duplicates to their assigned wells (final concentration will be half of the solution concentration).
4. 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.
5. Dilute 250 µL cell suspension with 5.75 mL Diluent.
6. Add 40 µL diluted cells to each well.
7. Place the lid on the plate, mix and incubate for 6 hours at 37 °C with 5% CO_2.

Adding substrate solutions

8. Equilibrate the plate and the substrate solutions to room temperature.
9. 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.
10. 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.

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Normalization

The reporter gene used for normalization, 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 signal with the Renilla normalization signal through simple division.

In the case of growth factors such as 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 (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.

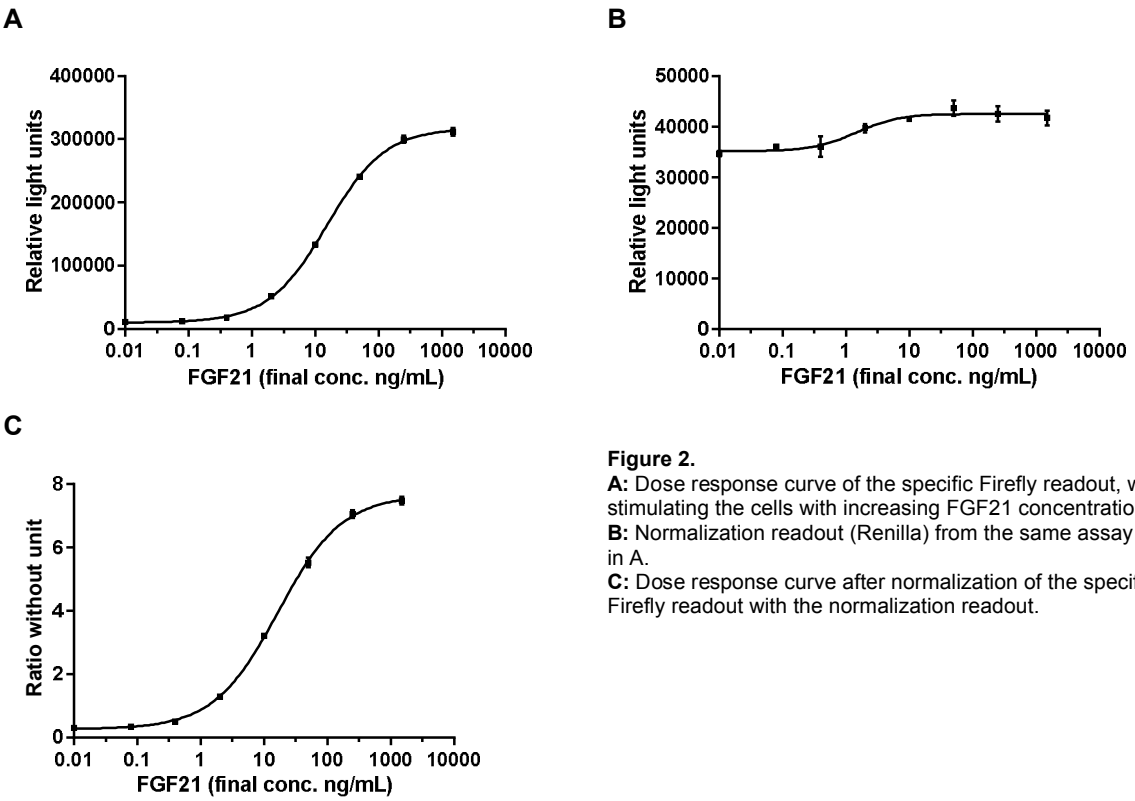

Figure 2.
A: Dose response curve of the specific Firefly readout, when stimulating the cells with increasing FGF21 concentrations.
B: Normalization readout (Renilla) from the same assay as in A.
C: Dose response curve after normalization of the specific Firefly readout with the normalization readout.

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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.

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

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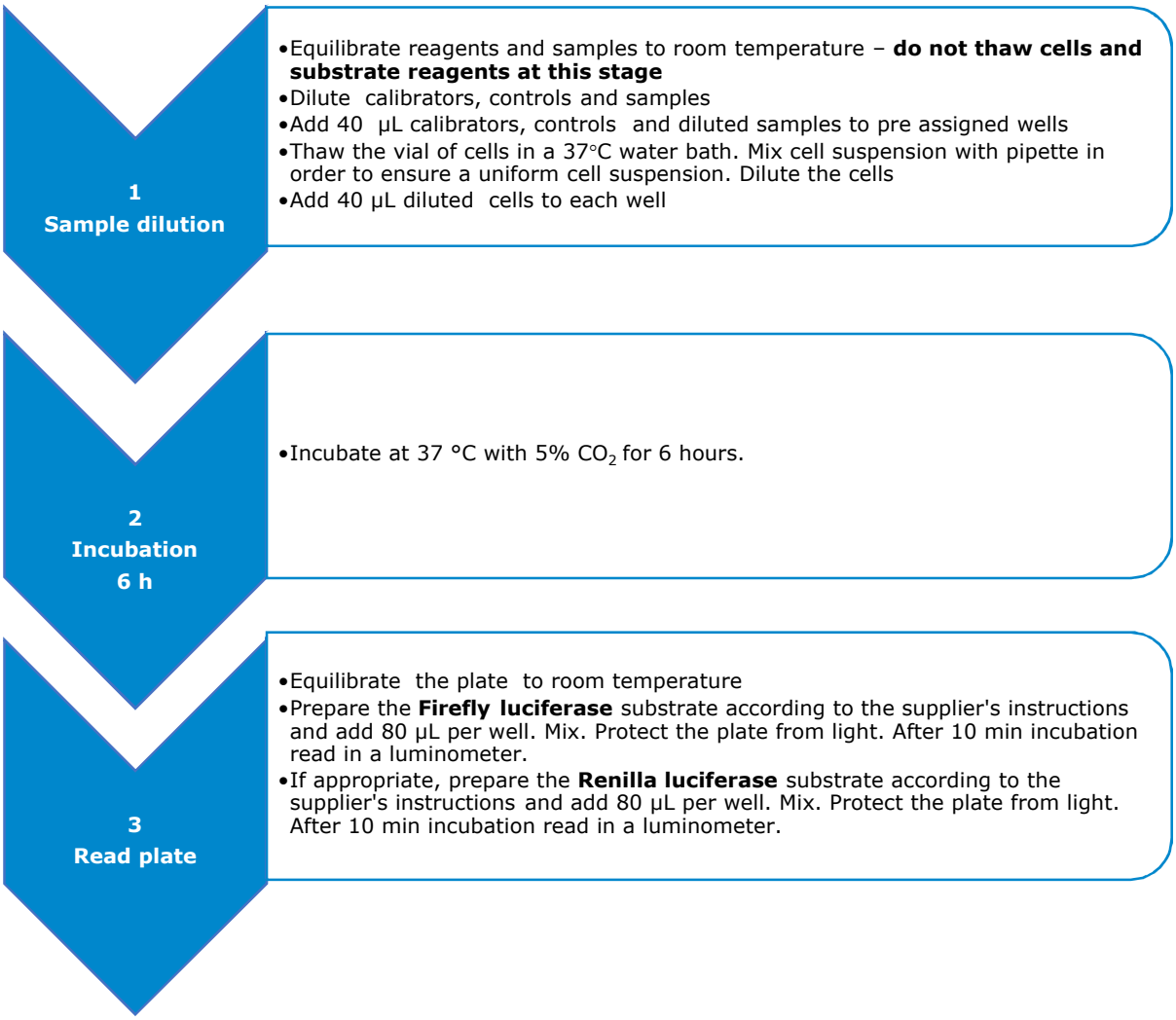

Troubleshooting and FAQ

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.