iLite® FGF21
ASSAY READY CELLS

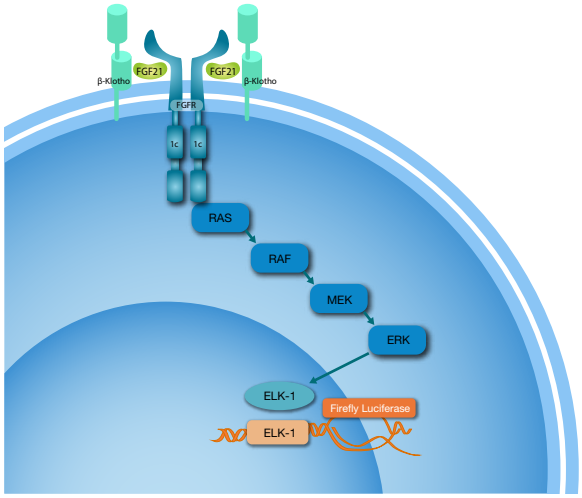
Research and development within metabolic disorders such as diabetes and obesity is a rapidly growing area. *iLite* FGF21 Assay Ready Cells have been designed to respond to one of the newer targets in this area, FGF21, with unparalleled sensitivity.

Human Fibroblast Growth Factor 21 (FGF21) is a member of a family of the atypical fibroblast growth factors, which can diffuse throughout the body and act as hormones. FGF21 acts mainly on FGF Receptor 1 (FGFR1) and stimulates glucose update into adipocytes, an effect which is additive with insulin, and redistributes fatty acids by lowering release of fatty acids from adipocytes and activation of Lipoprotein Lipase (LPL). As drug targets, there are a multitude of applications, e.g. both FGF21 analogues and FGFR1 antagonists are in development for treatment of insulin resistance and type 2 diabetes. Several FGFR1 antagonists are also in development for cancer treatment.

The *iLite* FGF21 Assay Ready Cells are a genetically engineered reporter gene cell line responsive to FGF21 by specific and proportional expression of Firefly Luciferase. The receptor chain FGFR1c is overexpressed on the surface together with the co-factor β-Klotho which has been genetically optimized to enhance sensitivity. Normalization of cell counts and serum matrix effects is obtained by a second reporter gene, a Renilla Luciferase reporter gene construct, under the control of a constitutive promotor.

For drug development in the field of metabolic disorders, *iLite* FGF21 Assay Ready Cells perfectly complements *iLite* Insulin Assay Ready Cells for measurement of potency and immunogenicity.

- Unparalleled sensitivity (EC50 10 ng/mL)
- High serum tolerance (no effect at 5% human serum)
- High reproducibility due to Assay Ready Format
- High fold induction (10x)



Schematic illustration of FGF21 binding to FGF receptor 1c (FGFR1c) in the presence of the genetically optimized co-receptor β-Klotho.

***iLite*® Insulin Assay Ready Cells**	
Product code	BM3071
Format	Assay Ready Cells
Related Products	BM3060 *iLite*® Insulin Assay Ready Cells
Application	The *iLite*® FGF21 Assay Ready Cells can be used to quantify FGF21 activity, FGFR1c inhibitor activity and for determination of neutralizing antibodies against such drugs in human serum. Application Notes for the following assays are available: • Quantification of FGF21 using *iLite*® FGF21 Assay Ready Cells • Determination of neutralizing antibodies against FGF21 using *iLite*® FGF21 Assay Ready Cells
Incubation time	Drug Assays 6 hours NAb Assays 30 min +6 hours
Detection system	Luminescence
Availability	Research Use Only (RUO)*

**These products are intended for professional research use only. The data and results originating from using the products, should not be used either in diagnostic procedures or in human therapeutic applications.*

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, and recipient is only to use them directly in assays. The 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 would constitute an infringement.

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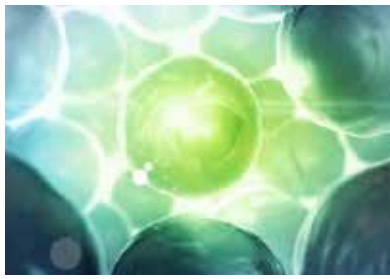
Answers in Life Science

FGF21 Responsive Reporter Gene Cell Line With Improved Sensitivity



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Abstract

A FGF21 responsive reporter cell line has been established that exhibits enhanced sensitivity to treatment with FGF21 by replacing the FGFR1c co-receptor β-Klotho with a codon optimized synthetic β-Klotho gene. This cell line allows the precise and rapid quantification of FGF21 activity even in the presence of human serum.

Introduction

Human fibroblast growth factor-21 (FGF21) has the potential to improve glycemic control in patients with type 2 diabetes, as well as the control of weight gain. Analogues of FGF21 are currently being tested in clinical trials in patients with type 2 diabetes and there is a need for a specific assay with enhanced sensitivity both for the quantification of the potency and neutralizing antibody response to FGF21 and related analogues in human serum. A reporter gene cell line has been established that exhibits a high degree of sensitivity to treatment with human FGF21 even in the presence of human serum. These cells can be used in a frozen assay-ready format that confers ease of use for both potency assays and neutralizing antibody assays for monitoring patients treated with FGF21 related products.

Methods

Human HEK293 or Jurkat cells were co-transfected sequentially, with a *Firefly* luciferase (FL) reporter-gene construct regulated by a chimeric transcription factor consisting of the trans-activation domain of Elk-1 fused to the GAL4 DNA binding domain (**Figure 1A**) together with an expression vector for the chimeric transcription factor. Since the GAL4 DNA binding domain does not exist in mammalian cells, only the chimeric transcription factor will bind to the up-stream activation sequence (UAS) of GAL4 regulating transcription of the FL reporter gene. The cells were also co-transfected with the gene encoding *Renilla* luciferase under the control of a constitutive promoter, used to normalize FGF21 induced FL activity, and the FGFR1c receptor chain together with a codon optimized synthetic co-receptor β-Klotho gene with 79% homology to the native gene (**Figure 1B**).

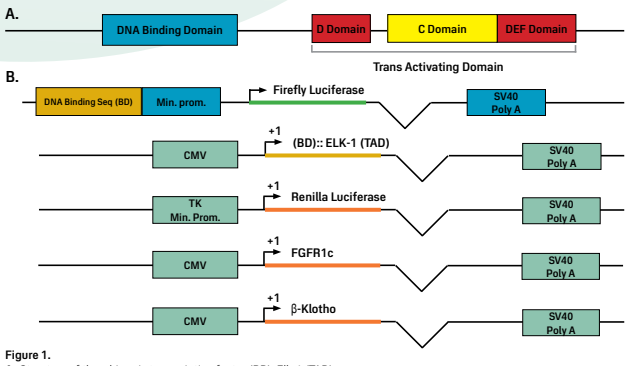

Figure 1.
A: *Structure of the chimeric transcription factor (BD)::Elk-1 (TAD)*
B: *Expression vectors introduced into HEK293 cells*

Results

I. Assay-Ready HEK293 cells with improved sensitivity to FGF21

Three different FGF21 responsive reporter cell lines (Jurkat, HEK293 native β-Klotho, and HEK293 optimized β-Klotho) produced in a frozen assay-ready format were thawed and incubated with increasing concentrations of FGF21 at 37°C, for 6 hrs prior to quantification of firefly luciferase activity. HEK293 cells transfected with the optimized β-Klotho gene exhibited enhanced sensitivity relatively to either HEK293 cells or Jurkat cells transfected with the native β-Klotho gene.

	HEK293 Optimized β-Klotho	HEK293 Native β-Klotho	Jurkat Native β-Klotho
Top (FL RLU)	**142702**	130408	40903
Bottom (FL RLU)	**13685**	1737	1530
Hill Slope	**1.216**	1.142	1.002
EC50 (ng/mL)	**7.055**	23.97	171.6
Span (FL RLU)	**129017**	128670	39373

Table 1. *Key characterizations of three FGF21 responsive cell lines produced in an frozen assay-ready format. Sigmoidal, 4PL, Best-fit values.*

II. The response of individual vials of frozen HEK293 cells (optimized β-Klotho) to treatment with FGF21

Vials of frozen assay-ready HEK293 cells (optimized β-Klotho) were thawed and treated with increasing doses of FGF21 for 6 hours at 37°C, prior to the quantification of luciferase activity using Dual Glo (Promega, Catalogue N° 2920) as shown in **Figure 2**. The assay-ready format confers ease of use while maintaining a high degree of sensitivity and specificity comparable to that of cells in culture.

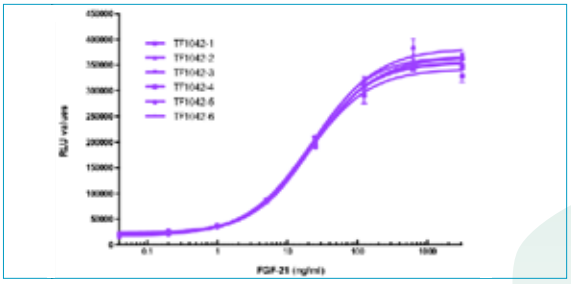

Figure 2. *Response of individual vials of assay-ready HEK293 (optimized β-Klotho) cells to treatment with FGF21*

III. Normal human sera have a minimum effect on the response of Assay-Ready Frozen HEK293 cells (optimized β-Klotho) to treatment with FGF21

Frozen assay-ready HEK293 cells (optimized β-Klotho) were thawed and treated with increasing concentrations of FGF21 in the presence of a 1/20 final dilution of human sera from normal doners at 37°C, for, 6 hrs. prior to quantification of firefly luciferase activity. (**Figure 3**). The response of FGF21 is only minimally affected by the presence of normal human sera, suggesting that the use of a chimeric transcription factor to regulate reporter gene expression markedly limits interference from other growth factors and cytokines present in normal human serum.

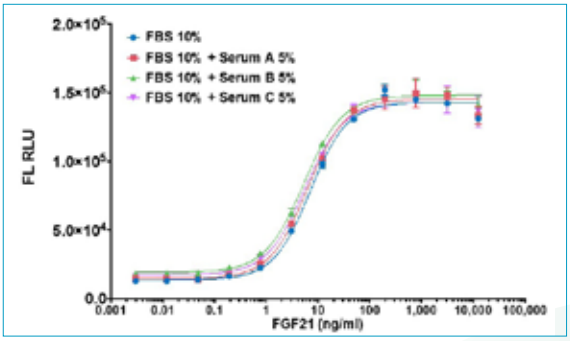

Figure 3. *Effect of normal human sera on the response of Assay-Ready HEK293 cells (optimized β-Klotho) to treatment with FGF21*

	FBS	Serum A 5%	Serum B 5%	Serum C 5%
Top	142702	145199	148008	142591
Bottom	13685	14600	18962	17186
Log EC50	0.8485	0.7957	0.7222	0.7456
Hill Slope	1.216	1.186	1.15	1.17
EC50 (ng/mL)	7.055	6.248	5.275	5.566
Span	129017	130599	129046	125405

Table 2. *Key characterizations of the FGF21 responsive cell lines when analysing three different serum samples. Sigmoidal, 4PL, Best-fit values.*

Conclusions

A Human β-Klotho gene was designed to use the codons most frequently employed statistically in order to optimize translation and hence increase the efficiency with which the β-Klotho co-receptor facilitates binding of FGF21 to its receptor and activation of signal transduction.

Synthesis of the corresponding gene and its use to transfect human HEK293 cells led to the establishment of a cell line that is markedly more sensitive than FGF21-responsive Jurkat or HEK293 cell lines previously established using the same cloning strategy but employing the native β-Klotho gene.

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