How to Select and Optimize Characterisation Criteria for Assay Ready Cells – A Case Study

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Answers in Life Science

INTRODUCTION

Characterization of cultured cell lines for production of biologic material and cell lines used for potency applications are outlined in various guidance documents – but there is an absence of specific instructive information about the requirements for genetically modified cell lines, such as modified cell banks used for production of assay ready cells utilized for e.g. in bioassays, ADCC assays and neutralization assays in GxP applications.

We have developed a technology based upon the Reporter Gene Assay format that allows for both the presence, activity and function of a chosen target/drug, inhibitors for such target/drug or neutralising antibodies against the drug to be assessed and quantified. It can be adapted for applications during the whole drug development such as drug discovery, quantification of drug potency, and for the analysis of functional drug levels and anti-drug neutralizing antibodies in clinical studies and post-market commitments.

Here we offer examples from 10+ years working in development, validation and production of these custom genetically modified cell lines and assay ready cells used in reporter gene assays.

TECHNOLOGY

iLite® technology is based upon a reporter gene assay format, modified and adapted for applications during the whole drug development continuum as well as for monitoring of biological drugs.

Briefly, host cells are transfected with a Firefly luciferase reporter gene construct. The reporter gene is under the control of drug responsive synthetic promoter which incorporates recognition sequences for specifically designed chimeric transcription factor(s) involved in relevant signal transduction pathways.

The reporter gene is thus only allowed to be activated by one specific pathway and by doing so “crosstalk” between the transcription factors can be avoided, rendering a more specific assay. The cells also contain the Renilla luciferase reporter gene under the control of a constitutive promoter, that allows the target induced firefly luciferase activity to be normalised relative to the Renilla luciferase expression. This provides a means for correcting for serum matrix effects and differences in cell viability. The read-out is light emission as measured by a standard luminometer.

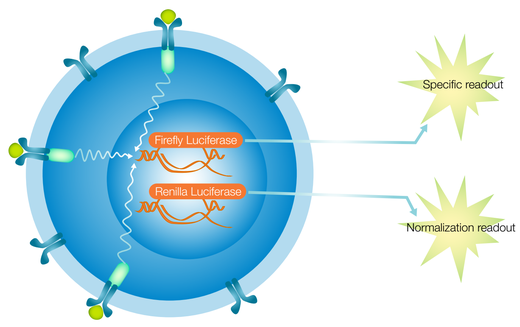


Figure 1. Schematic illustration of the *iLite* reporter gene cell.

iLite® FGF21 – CELL LINE DEVELOPMENT:
FROM CLONE SELECTION TO PRODUCT MANUFACTURING

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.

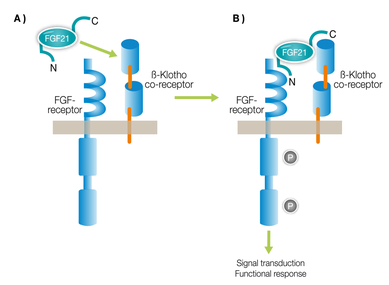


Figure 2. Schematic illustration of FGF21 binding to FGF receptor 1c (FGFR1c) in the presence of the genetically optimized co-receptorβ-Klotho

CELL SELECTION

When selection a cell line, identification of components of the signaling pathway such as receptor, transcription factor, gene reporter is of great importance as well a transfection construct and transfection dilutions,

Furthermore, testing of critical reagents for cell growth, viability and doubling time to set future cultivation time / number of passages for manufacturing of *iLite* FGF21 ARC is key to establish a reproducible product.

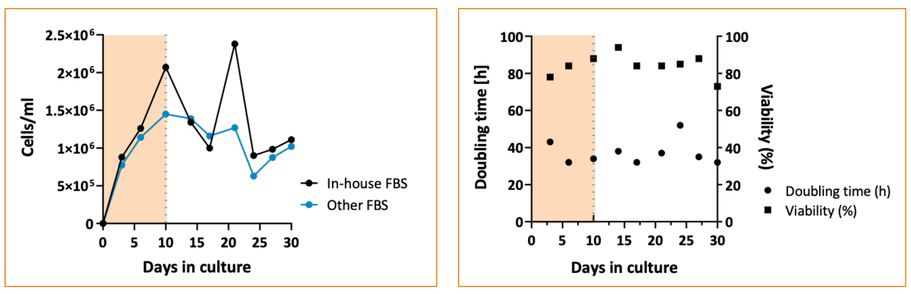
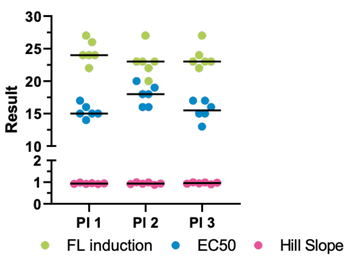


Figure 3.

Testing of critical reagents for cell growth, viability and doubling time to set future cultivation time / number of passages for manufacturing of *iLite* FGF21 Assay Ready Cells

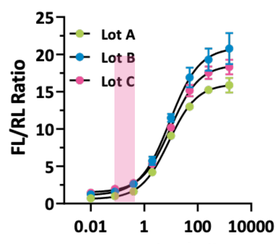
ASSAY VARIATION



Assay variation	Plate 1	Plate 2	Plate 3	CV%
Mean EC50	16	18	15	9,2
Mean FL induction	25	23	24	3,3
Hill Slope	0,94	0,94	0,95	0,88

Figure 4a.

Evaluation of the day-to-day and operator-to-operator including 6 vials of assay ready cells per plate, analyzed in two replicates. In total three plates on two different days performed by two different operators where analyzed.



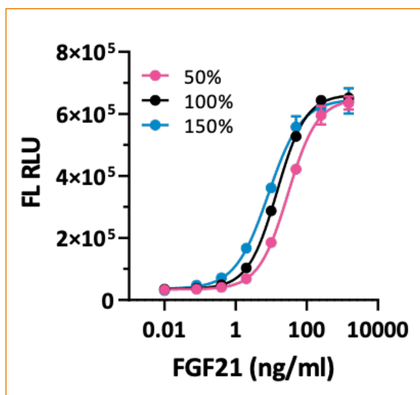
Assay variation	Mean	%CV
EC50	9,2	5,62
FL induction	16,6	14,5
Hill Slope	0,89	0,72

Sample ID	FGF21 (ng/mL)	CV%	Accuracy (%)
1	39,9	11,1	99,6
2	38,5	17,4	110
3	26,1	1,47	87,0

Figure 4b.

Testing of lot-to-lot variations, measuring three different lots of *iLite* FGF21 cells by analyzing the dose-response curve as well as technical samples in 3 replicates.

BIOASSAY APPLICATION CHARACTERIZATION - POTENCY



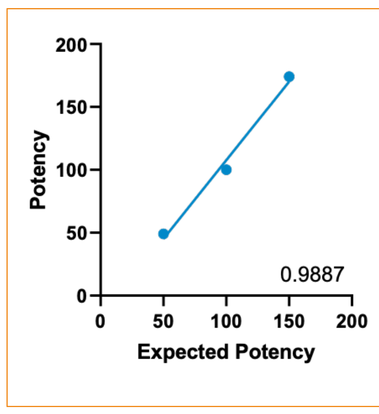


Figure 5.

Evaluation of potential application for drug potency determination

- Accuracy: 101-115%

CONCLUSION

We have 10+ years working in development, validation and production of custom genetically modified cell lines and assay ready cells used in reporter gen assays. The lessons learned from this extensive work, and our recommendations on what can be considered as an appropriate level for assay read cells characterisation and suggested applications are:

- The *iLite*® technology eliminates many of the limitations of conventional cell-based assays since The cells are delivered as assay ready frozen cells allowing cell-based assays to be carried out without the need for cell culture or the maintenance of cells continuously in the laboratory and enable same day results.
- We recommend that cell lines are identified and screened for key aspect such as specificity, receptor expression, cell growth and signal induction performance.
- Risk based approach to decide extent of characterisation/validation needed, including critical reagents is highly favourable and should be used as a routine
- Qc trend and real time stability indicates that this cell can be stored for +30 months at -80°C
- Applicable to be used for Potency assessments of biologics and for detecting neutralizing antibody against biologic therapeutic compound

All in all, this learnings suggest that the *iLite*® Assays Ready Cell lines can give a significant cost reductions and increases the applicability of cell-based assays to routine use as potency or neutralization assays.

CELL LINE VALIDATION: QC TREND AND STABILITY

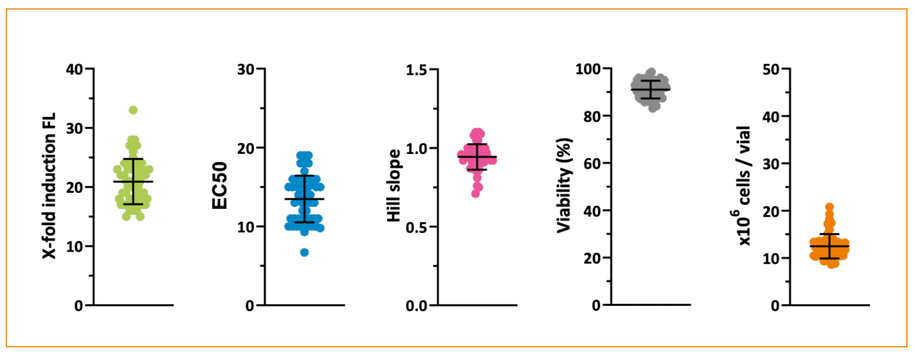
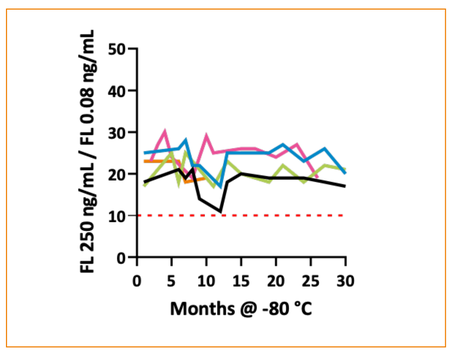


Figure 6a.

Record of the QC trend including FL induction, EC50, Hill Slope, Viability and number of cells / vial of *iLite* FGF21 assay ready cells.

Data were collected from 9 different lots with 6 vials per lot analyzed.



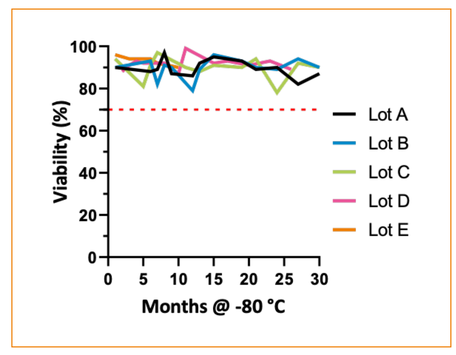
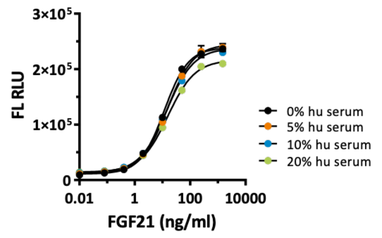


Figure 6b.

Real-time stability study of FGF21 assay ready cells over 30 months at -80°C with 5 different lots.

The stability testing will be continued until 36 months after production

CLINICAL IMMUNOGENICITY CHARACTERIZATION



Serum concentration	0%	5,0%	10%	20%
Hill Slope	1,00	0,93	0,89	0,89
EC50	11,5	15,1	14,2	15,6
FL induction	17,7	15,9	14,6	12,6

Figure 7a.

FGF21 dose response curve prepared at different concentration levels of human serum pool, from 0 to 1000ng/ml FGF21

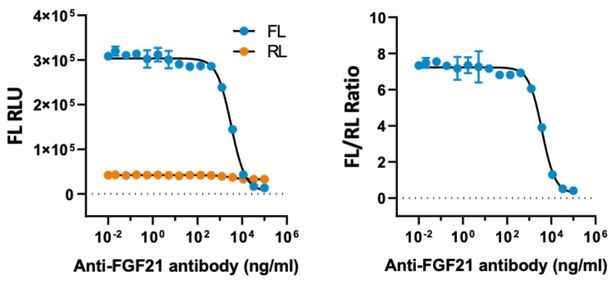


Figure 7b.

Evaluation of NAb assay application using an anti-FGF21 antibody in present of 55 ng/ml FGF21. A > 20-fold signal reduction of the firefly signal indicates room to improve nab assay sensitivity, altough indicating the detection of neutralizing antibody directed against FGF21 at low level of FGF21 in the patient sample is feasible.