Outline for Characterization of Reporter Cell Lines

Critical Reagents for Robust Cell-based Reporter-Gene Assays

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Answers in Life Science

INTRODUCTION

Over the last decades, biopharmaceuticals have revolutionized the treatment of a wide range of diseases in all areas of medicine. The development of such drugs has led to a need for bioassays that can be used to accurately characterize various aspects of the drug. Developing a bioassay is a complex, rigorous undertaking that needs to address several challenges including modeling the mechanisms of action associated with the biotherapeutic and ensuring that robust and validatable assays can be achieved.

Assay-ready reporter-gene assays have proven to be a valuable tool in assessing both functionality and MOA-representing assays, but given their importance in drug development, reporter-gene assays need to be designed, produced, and validated in a way that ensures that they behave consistently over time, in varying experimental setting and subsequent pivotal assays.

Here, we present an outline for a suggested set of assays by which the performance of cell-based reporter gene assays can be determined and validated.

This is an essential step in the quality assessment to ensure that the assays are reliable, accurate, and reproducible and that errors resulting from biological variation and methodology are kept to a minimum.

MATERIAL & METHODS

We have assessed the performance of a number of assay-ready reporter-gene cell lines as exemplified here using the *iLite*® IL-23 responsive assay-ready cells. Similar data is available for a suite of *iLite* Assay Ready cells.

The *iLite*® cell-based assays are based on a simple reporter gene technology. Receptors, specific for a certain target or ligand, are expressed on the surface of a cell. Once the ligand binds to the receptor, this will trigger an intracellular signaling cascade, which leads down to a promoter region, fused to the reporter gene, in this case, the Firefly Luciferase. Activation of the reporter gene along with the addition of a substrate will generate light (Fig. 1).

The amount of light will be correlated to the amount and activity of the ligand that was bound to the receptors.

All assays were performed according to the manufacturer's instructions and using assay-ready since they are shown to have inherent advantages such as:

- **Stable cell phenotypes and passage numbers**
- **Offering lower performance variation**
- **Reduced assay times**
- **More homogenous and optimized workflow**

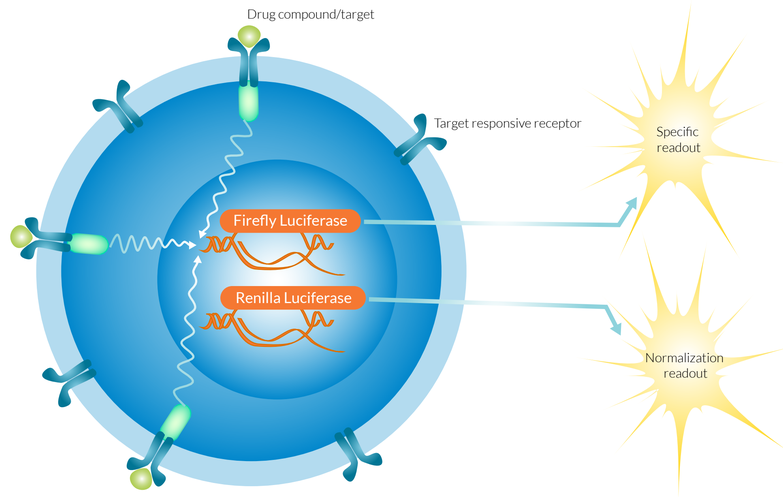


Fig 1. *iLite cell-based technology principle*

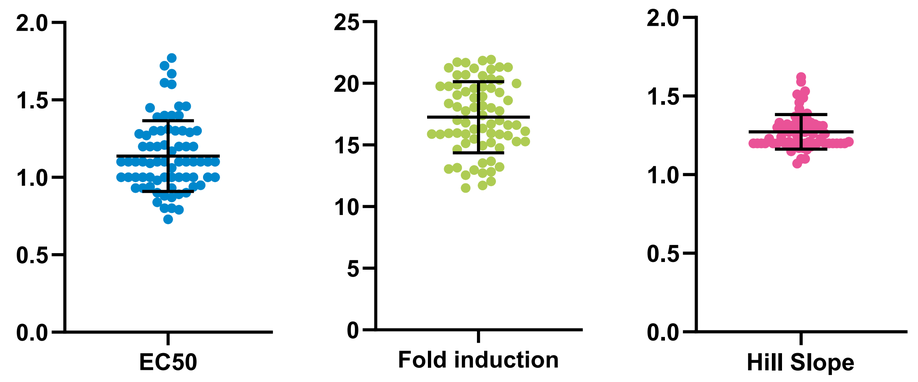
CELL LINE VALIDATION

Our procedures for establishing a new cell line include several pre-determined steps:

- Cell line validation
- Risk assessment
- Production of 3 validation batches
- Lot-to-lot variation
- Day-to-day variation
- Operator variation
- QC-limits (EC50, Hill slope, Fold induction)

Assay variable	Mean	CV %
EC50	1.1	20
FL induction	17	8.7
Hill Slope	1.2	17

Key performance parameters such as - EC50, Fold induction and Hill slope - **were determined using technical samples.**



Cell line variation

Data were collected from tests with an IL-23 functional bioassay analyzing a dose-response curve covering a concentration range from 0-63 ng/ml IL-23.

The data represent results from 71 dose-response curves using 5 different cell batches performed by 3 operators at 11 different days.

Fold induction was calculated by: Firefly Luciferase RLU 21 ng/ml/FL, RLU 0.086 ng/ml. CV; coefficient of variation.

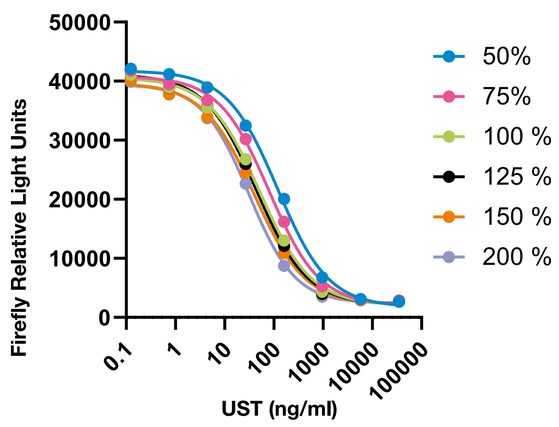
BIOASSAY APPLICATION CHARACTERIZATION

We also perform an overall Bioassay Characterization – where accuracy and the variance between days and lots is determined.

To assess the impact of any variance on the actual bioassays, the following key performance aspects are assessed:

- Initiation of real-time stability
- Assay sensitivity
- Potency
- Intra- and inter-assay precision
- Homogeneity
- Accuracy

An important aspect of any Linearity/Potency assay is to establish the assay's ability to provide measured values proportional to the sample concentration.



Potency

The iLite anti-IL-23 inhibitory bioassay was performed with one reference dose-response curve, called 100% and with concentrations at 50, 75, 125, 150 and 200% of the reference UT; Ustekinumab.

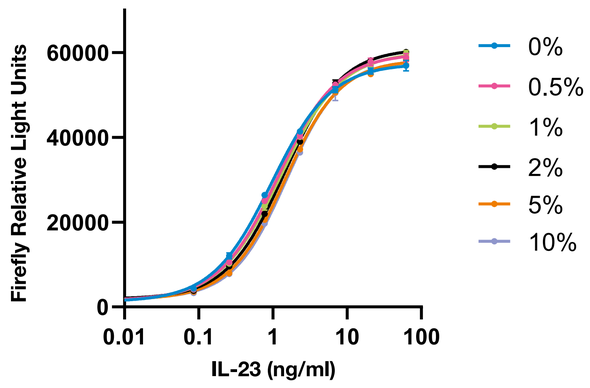
Expected potency	50%	75%	100%	125%	150%	200%
IC50	119	80.0	51.3	43.8	39.5	31.0
Accuracy	86%	86%		94%	87%	83%
Hill Slope	-0.839	-0.850	-0.837	-0.837	-0.837	0.920
FL reduction	14.2	12.5	17.2	15.9	15.4	14.7

CLINICAL IMMUNOGENICITY CHARACTERIZATION

These assays are more complex since they are performed using clinical samples with complicated matrixes and therefore the following aspects need to be taken into consideration:

- Serum tolerance
- Drug tolerance
- Assay sensitivity
- NAb assay sensitivity
- Cut point evaluation
- Repeatability
- Plate-to-plate variation
- Homogeneity

Assays to determine how the composition of the samples, i.e., the serum tolerance affects the assay outcome.



Serum effect/tolerance.

iLite IL-23 functional assays were performed with increasing amounts of human

Serum concentration	0%	0.5%	1%	2%	5%	10%
Hill Slope	1.07	1.08	1.05	1.12	1.15	1.19
EC50	0.96	1.17	1.26	1.41	1.46	1.52
FL induction	13	14	14	15	15	17

CONCLUSION

Assay-ready reporter-gene assays are essential tools in the development of biopharmaceuticals. Their important functions as a critical reagent in many pivotal bioassays mean they must perform well and consistently over time and under different experimental settings. Performance assessment and characterization is there for key.

The aspect evaluated can be divided into three main themes:

- **Cell line validation**
- **Bioassay Application Characterization**
- **Clinical Immunogenicity Characterization**

Here, we have presented a set of procedures that can be used to assess the performance of assay-ready reporter-gene assays and subsequent results we have obtained using the *iLite* Functional Bioassays. Following these guidelines in developing and subsequently characterizing cell-based bioassays ensures that the assay can generate consistent data over time and in different environments.

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