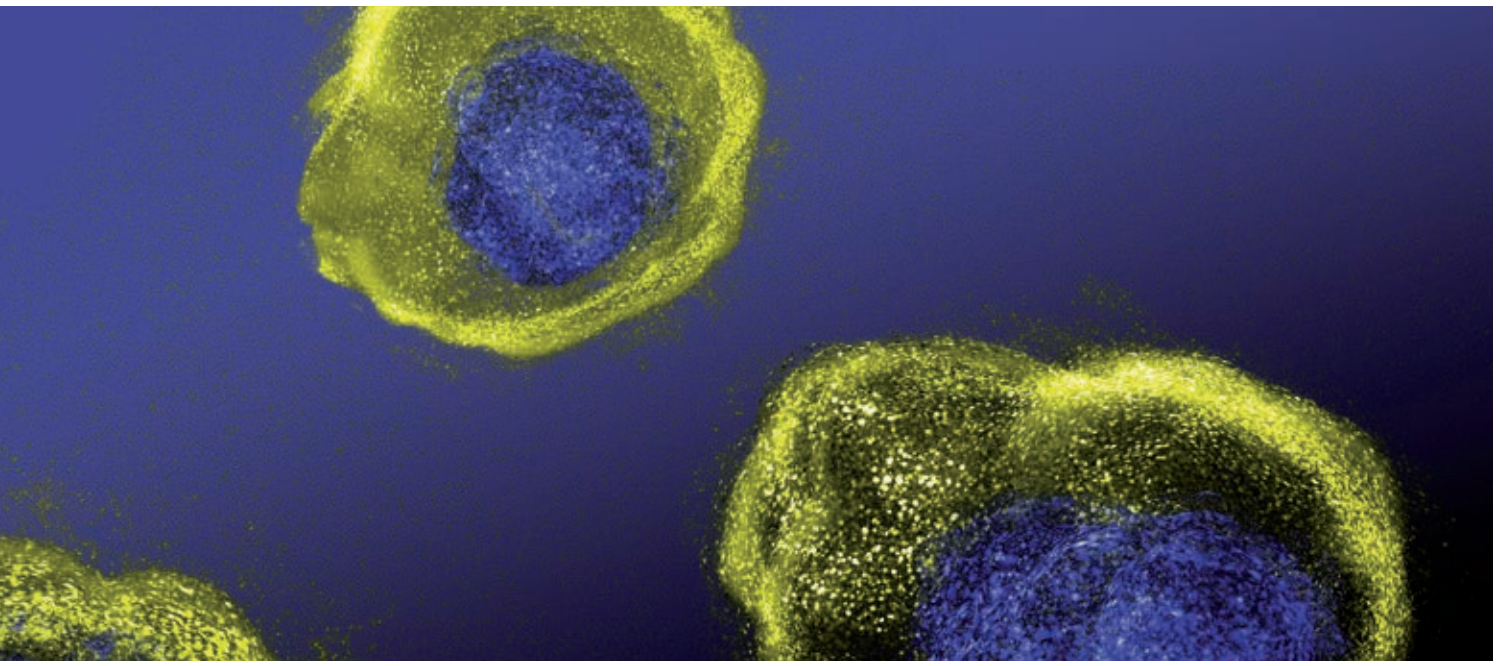


iLITE® IL-12 PERFORMANCE DATA

A QUALIFICATION OF THE MOST COMMON BIOANALYTICAL ASPECTS OF A CELL-BASED REPORTER GENE ASSAY

INTRODUCTION TO *iLITE* REPORTER GENE TECHNOLOGY

Cell-based *iLite*® assays are cleverly designed, sensitive and specific reporter-gene assays with firefly luciferase readout. Most assays also contain a secondary reporter-gene that can be used for normalization purposes.

They can be developed for virtually any pharmaceutical target and allow an easy, rapid and accurate test format for a wide range of applications – including measurement and quantification of drug activity, immunogenicity and ADCC activity.

Here, we present performance data for the *iLite* IL-12 cell-line. The generation of such data is an important 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.

iLITE® CELL-BASED SOLUTIONS - IL-12 ASSAY READY CELLS

The *iLite*® IL-12 Assay Ready Cells are a genetically engineered reporter gene cell line responsive to IL-12 by specific and proportional expression of Firefly Luciferase.

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. The *iLite* IL-12 Assay Ready Cells can be used for the quantification IL-12 or p40 inhibitor activity, or for detecting a neutralizing antibody response against such inhibitors in human serum.

iLite IL-12 Assay Ready Cells is the perfect complement to the *iLite* IL-23 Assay Ready Cells, allowing testing for specific IL-12 inhibitory activity and screening for unwanted inhibitory activity of IL-23.

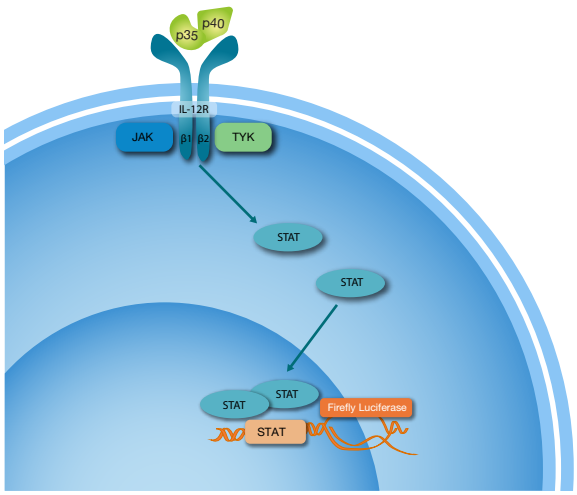


FIG. 1 - SCHEMATIC ILLUSTRATION OF IL-12
Schematic illustration of the over-expression of IL-12 receptor on the cell surface, which has no cross-reactivity with IL-23.

VALIDATION OF MANUFACTURING PROCESS & DETERMINATION OF THE OVERALL CELL LINE VARIATION

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

- Risk assessment to identify any potential hazards to the operating personnel, the environment or regarding the product as a whole
- Development phase with process optimization
- Manufacturing of at least 3 validation batches
- Assessment of performance criteria such as EC50, and Hill slope, X-fold induction to determine the overall cell line variation

Assay variable	Mean	CVC
EC50	0.41	29
FL induction	20	21
Hill Slope	1.1	5.0

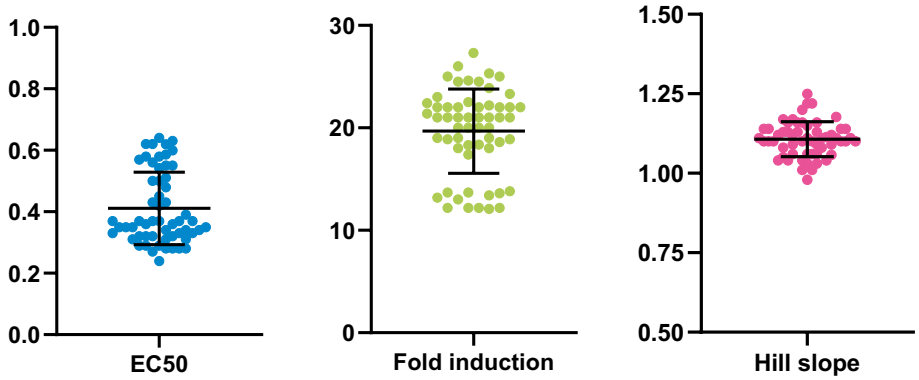


FIG. 2 - OVERALL CELL LINE VARIATION

Data were collected from tests with an IL-12 functional bioassay analyzing a dose-response curve covering a concentration range from 0-28 ng/ml IL-12. The data represent results from 53 dose response curves using 5 different cell batches performed by 3 operators at 12 different days. Fold induction was calculated by: Firefly Luciferase RLU 6.9 ng/ml/FL RLU 0.0068 ng/ml. CV; coefficient of variation.

REAL TIME STABILITY STUDY

Another important parameter is the stability of the cell line over time. We perform real time stability studies where the **defined QC parameters are monitored over time**. Our goal is to exceed a shelf life of 36 months on all our Assay Ready Cell lines, when stored at -80°C.

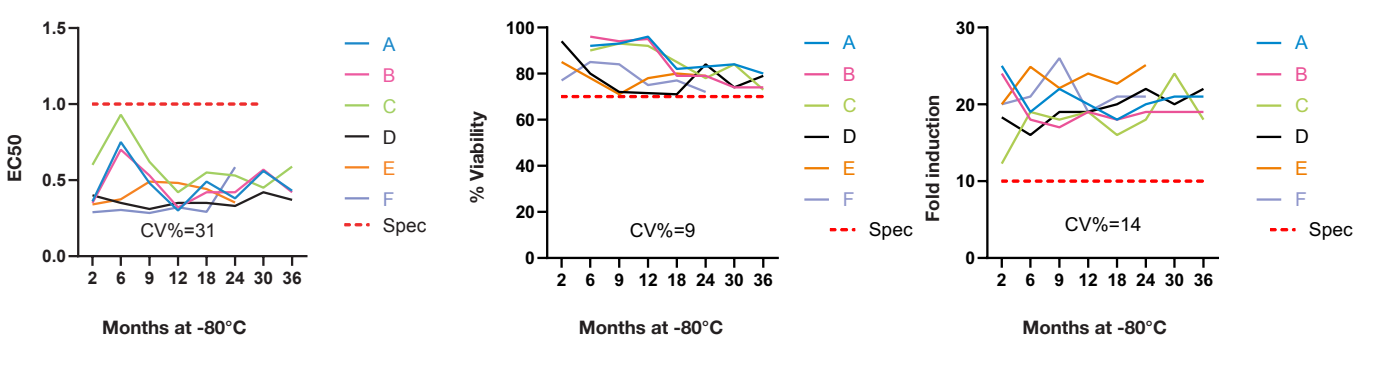


FIG. 3 - REAL TIME STABILITY AT -80°C.

The IL-12 assay ready cell line was tested regulary over 36 month by performing an IL-12 functional bioassay analyzing dose-response curves with cell batches manufactured at different time points.

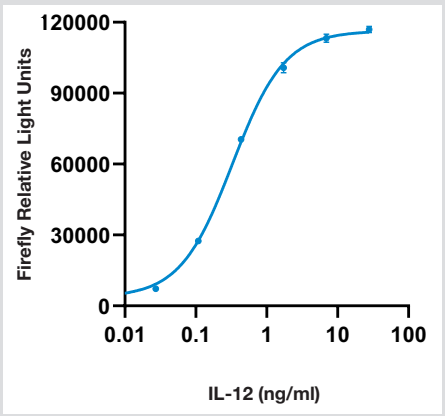
DYNAMIC RANGE

ASSAY APPLICATION EXAMPLE

When validating the cell lines, different potential applications are assessed using a standardized stimuli or commercial drug compound. The cell lines are evaluated based on:

- **Functional Dose-Response Range**
  The dynamic range of the assay is determined by performing a dose-response curve from which we establish the range of the assay, i.e., where we see a signal from the inducible Firefly luciferase reporter gene, measured in this case in Relative Light Units (RLU).
- **Inhibition Dose-Response Range**
  In the presence of inhibitory activity against the target, the amount of free target is reduced, resulting in a decreased stimulation of Firefly luciferase expression. Accordingly, the Firefly luciferase signal is inversely proportional to the amount of inhibitory activity in a sample.
- **Anti-Inhibitor (Neutralizing Antibodies, NAb) Dose-response Range**
  The Firefly luciferase signal is inversely proportional to the amount of inhibitory activity in a sample. In the absence of target inhibitor activity and suspected NAb presence in a test sample, a known amount of drug is added to quench the Firefly luciferase signal, resulting in a partially restored signal which can be used as a measure of the presence of NAbs in the sample.

DYNAMIC RANGE

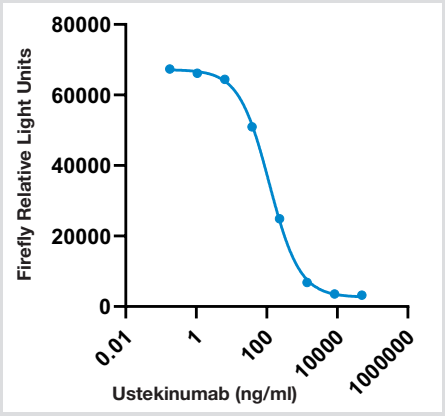


IL-12	In-assay (ng/ml)
A	28
B	6.9
C	1.7
D	0.43
E	0.11
F	0.027
G	0.0068
H	0

FIG. 4a - DYNAMIC RANGE

Example of a functional IL-12 bioassay application, were 0-28 ng/ml IL-12 was used to achieve a dose-response curve.

DYNAMIC RANGE - INHIBITION



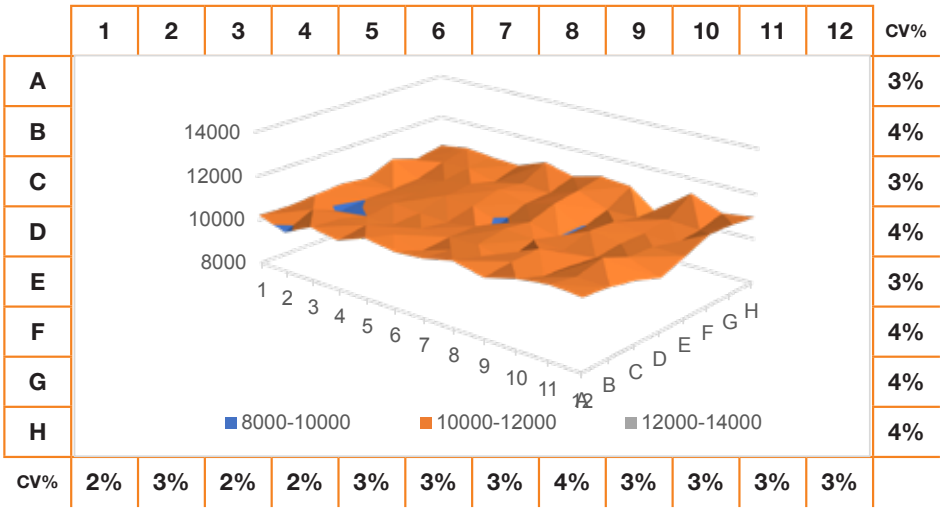
Final IL-12 6 ng/ml	UST In-assay (ng/ml)
A	100 000
B	16 667
C	2 778
D	463
E	77.2
F	12.9
G	2.1
H	0

FIG. 4b - DYNAMIC RANGE - INHIBITION

Example of an inhibitory IL-12 bioassay application, were 0-100.000 ng/ml IL-12 inhibitor was used with 6 ng/ml IL-12 to achieve a dose-response curve.

PLATE HOMOGENEITY

For optimal results, it is important to use a plate design for the readout that provides the optimum conditions for consistency, minimizing edge effects and to use opaque plates when using substrates that produce fluorescent signals.



Readout of this type can be prone to common errors such as edge effects or inherent inconsistency of the actual cells.

With the *iLite* Assay Ready Cells, we see very low variance across the plate, allowing the entire plate layout to be used in the plate design.

FIG. 5. HOMOGENEITY

One single sample, 0.05 ng/ml IL-12, representing the lower part of the linear range of the functional bioassay, was tested in all 96 wells of the plate.

2. BIOASSAY VARIATION AND PERFORMANCE

In addition to characterizing the cell line, validating the manufacturing process and creating Application Notes, we also perform an overall Bioassay Characterization – where accuracy and variance, between days and lots are determined.

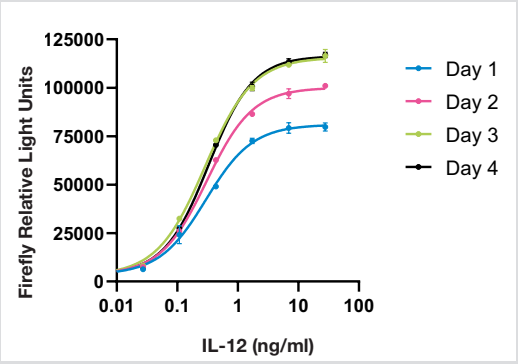
To assess the impact of any variance on the actual bioassays, key performance parameters such as:

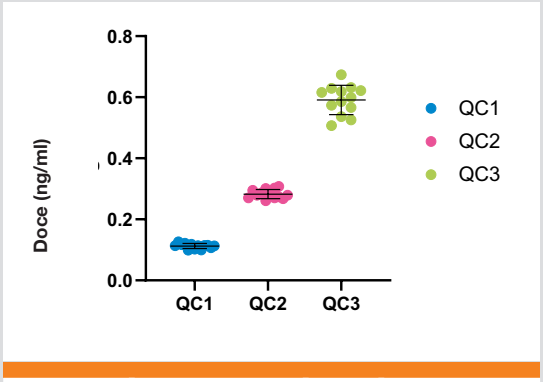
- EC50
- Fold induction
- Hill slope

are determined using technical samples. All this is performed to establish the necessary information regarding how they perform in their designated assays/applications.

ACCURACY & VARIATIONS – DAY-TO-DAY

The following assays are performed to determine how the results of the assay varies over time. This is important to ensure that the results are reproducible and that there is no drift in the results over time.





Assay variable	Mean	CV%
EC50	0.31	5.6
FL induction	20	10
Hill Slope	1.2	3.4

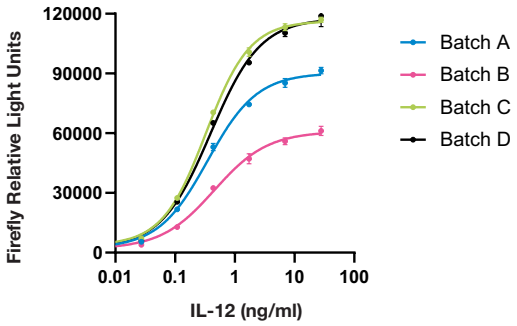
Sample ID	IL-12 (ng/mL)	CV%	Accuracy (%)
1	0.15	3.4	77
2	0.3	2.7	97
3	0.75	3.8	81

FIG. 6 - DAY-TO-DAY

The iLite IL-12 functional bioassay was performed with a dose-response curve and three technical samples with known amount of IL-12. Data shown is generated from assays performed at four separate days with the same batch of IL-12 cells.

ACCURACY & VARIATIONS – LOT-TO-LOT

Another important accuracy measurement is the variance between lots, to ensure a consistent readout of the assays over a longer period of time and for multiple lots.



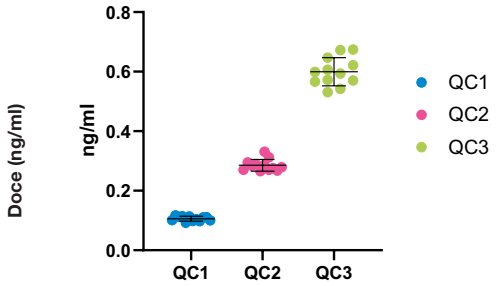


FIG. 7 - LOT-TO-LOT

The iLite IL-12 functional bioassay was performed with a dose-response curve and three technical samples with known amount of IL-12. Data shown is generated from using four different batches of IL-12 cells at

Assay variable	Mean	CV%
EC50	0.38	14
FL induction	22	11
	1.07	8.3

Sample ID	IL-12 (ng/mL)	CV%	Accuracy (%)
1	0.15	7.6	71
2	0.3	4.4	95
3	0.75	5.3	80

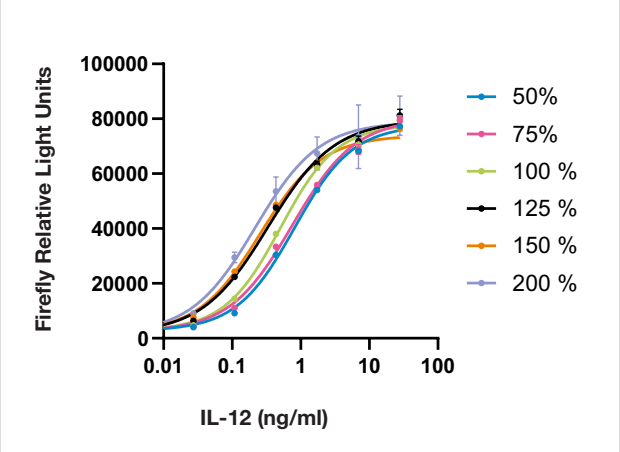
As shown in the figure, the precision and accuracy of the technical samples are well within acceptable limits, with CVs generally below 10%, even though the absolute Firefly Luciferase (FL) signal may vary. These results show that there are only small differences between assays performed on different days or using different batches, which is most probably due to the closely controlled production process and the assay ready format of the cells.

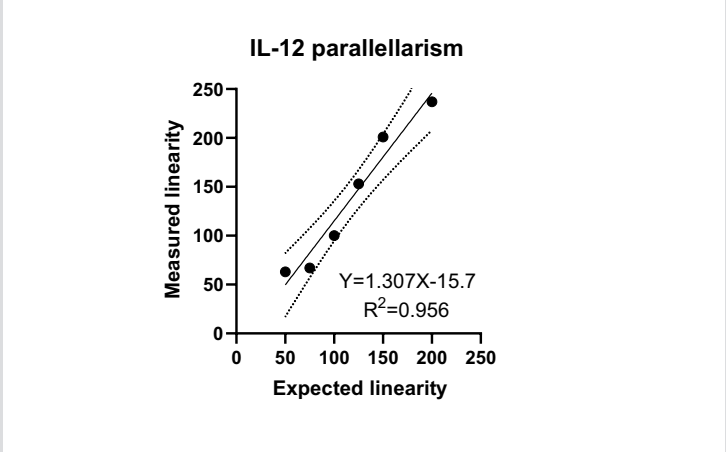
LINEARITY (PARALLELISM) FUNCTIONAL / LINEARITY (PARALLELISM) INHIBITORY

To show linearity between ligand/inhibitor concentrations and induced firefly expression, we perform a linearity assay using different starting concentrations of the ligand/inhibitor. The results are plotted against a 100% reference curve, which can be used to calculate the linearity between the expected EC50/IC50 and the measured EC50/IC50.

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

LINEARITY (PARALLELLARISM)



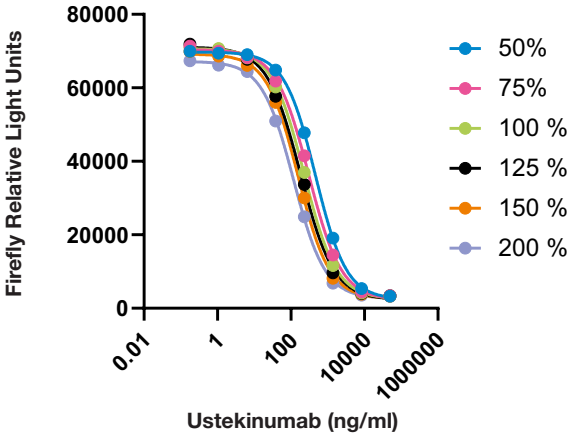


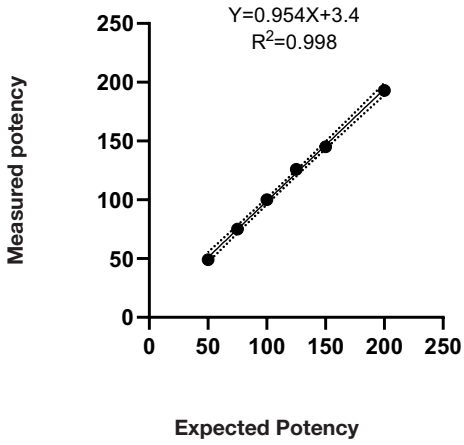
Expected linearity	50%	75%	100%	125%	150%	200%
EC50	0.809	0.757	0.510	0.334	0.254	0.216
Accuracy	126%	90%		122%	134%	118%
Hill Slope	1.02	0.944	1.059	0.900	0.959	0.929
FL induction	17.7	17.6	17.6	17.8	18.0	18.3

FIG. 8 - PARALLELISM / LINEARITY

The iLite IL-12 functional bioassay was performed with the one reference dose-response curve, called 100% and with concentrations at 50, 75, 125, 150 and 200% of the reference.

POTENCY





Expected potency	50%	75%	100%	125%	150%	200%
IC50	466	304	228	181	157	118
Accuracy	98%	100%		101%	97%	97%
Hill Slope	-1.031	-0.975	-0.991	-0.956	-1.015	-1.008
FL reduction	12.5	13.4	15.7	15.6	16.8	17.0

FIG. 9 - POTENCY

The iLite anti-IL-12 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 UST; Ustekinumab.

IMMUNOGENICITY CHARACTERIZATION

Assessment of undesirable immunogenicity of therapeutic proteins and peptides is a key element in biological drug development. These assays are more complex since they are performed using clinical samples with complicated matrixes and therefore need to reflect the in vivo mechanism of actions of the drug.

With our cell-based functional bioassays, screening for NAbs is a key application area. Here, it is important to determine how the composition of the samples, i.e., the serum tolerance, affects the assay outcome.

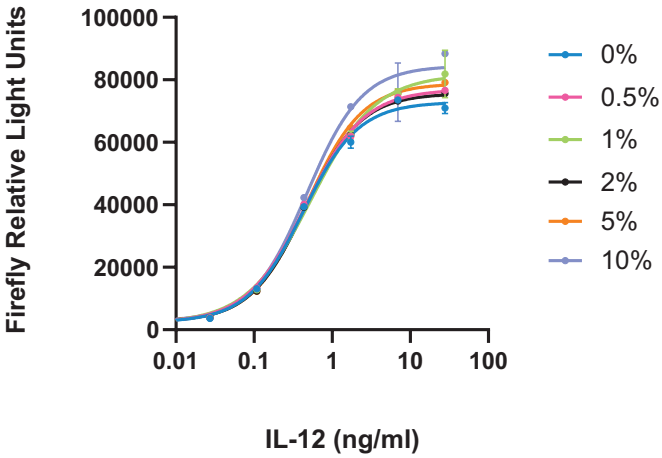
Two major parameters influence the outcome and robustness of an assay:

Nab assays Sensitivity

In order to determine the levels of neutralizing antibodies, a fixed concentration of the target is mixed with a clinical sample containing antibodies against the target. Upon the addition of increasing amounts of anti-target antibodies, the signal is gradually quenched and an inverted dose-response curve can be plotted.

Serum tolerance

To establish if the serum matrix with the clinical samples has any effect on the outcome of the assay, experiments were performed using a range of serum concentrations. This allows us to determine the effect of serum on the assays and establish the maximum serum concentration (tolerance) for the assay.



Serum concentration	0%	0.5%	1%	2%	5%	10%
Hill Slope	1.2	1.2	1.0	1.2	1.2	1.2
EC50	0.42	0.46	0.54	0.46	0.48	0.47
FL induction	23	23	22	21	22	21

FIG. 10 - SERUM EFFECT / TOLERANCE

The iLite IL-12 functional bioassay was performed with a dose-response curve including human serum, up to 10%.

Svar Life Science

Svar is a Swedish life science company that invents, develops and applies the best assay technology for drug development and clinical diagnostics with the goal of delivering new solutions tailored to customer requirements.

We establish practical platforms for clinical routine testing, work to secure relevant competences by sharing best practices and knowledge and our partnerships enable us to deliver flexible solutions depending on specific needs.

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