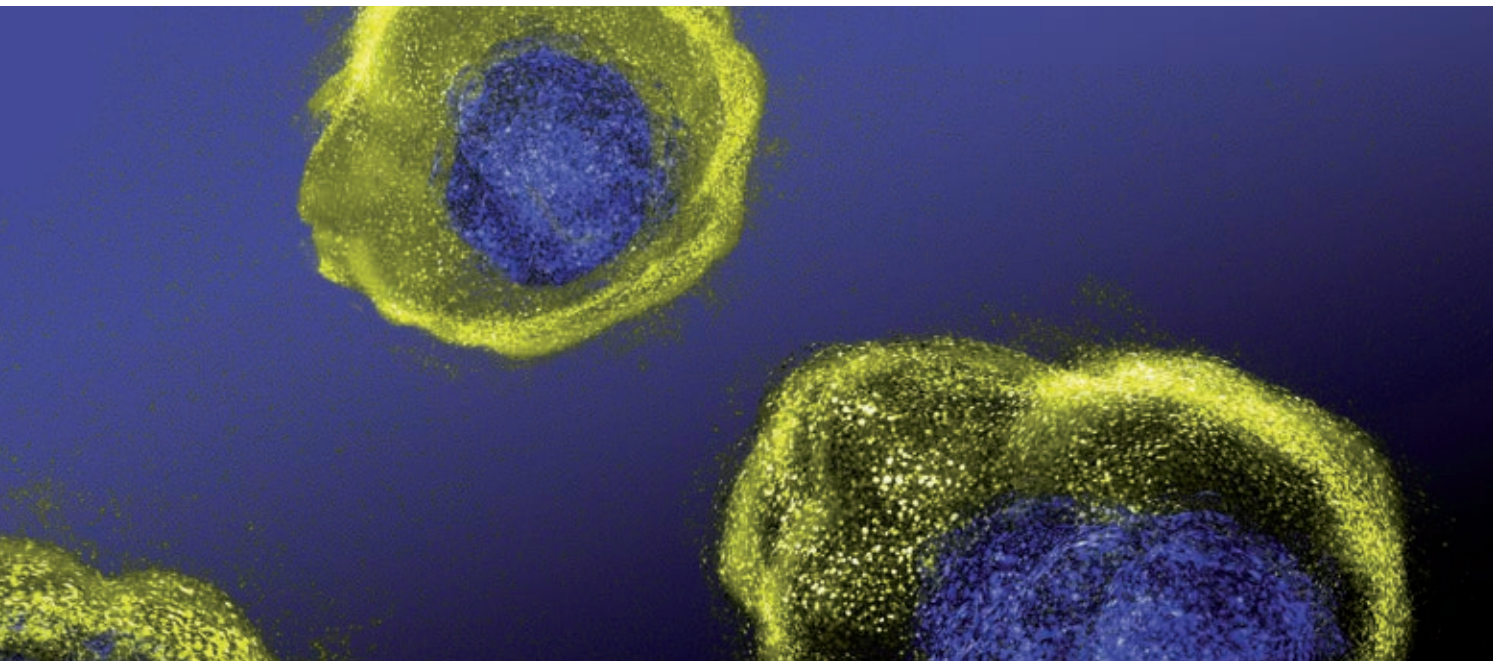


iLITE® ADCC CD20 PERFORMANCE DATA

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

iLITE ADCC REPORTER GENE ASSAYS

The surge in interest in developing therapeutic antibodies has led to increased demand for reliable cell-based assays to assess the ability of mAbs to elicit ADCC activity.

iLite cell-based ADCC functional assays use engineered reporter-gene-carrying effector cells in conjunction with target cells that express the specific antigen at constant high levels. By overexpressing the surface antigen, *iLite* target cells result in dramatically increased ADCC activity compared to the wild-type target cells used by competing systems. Combined with *iLite* effector cells that have been cleverly engineered to resemble the natural Fc signal transduction pathway, a powerful and very sensitive system is created.

Here, we present performance data for *iLite* ADCC effector cells combined with CD20 target cells. 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.

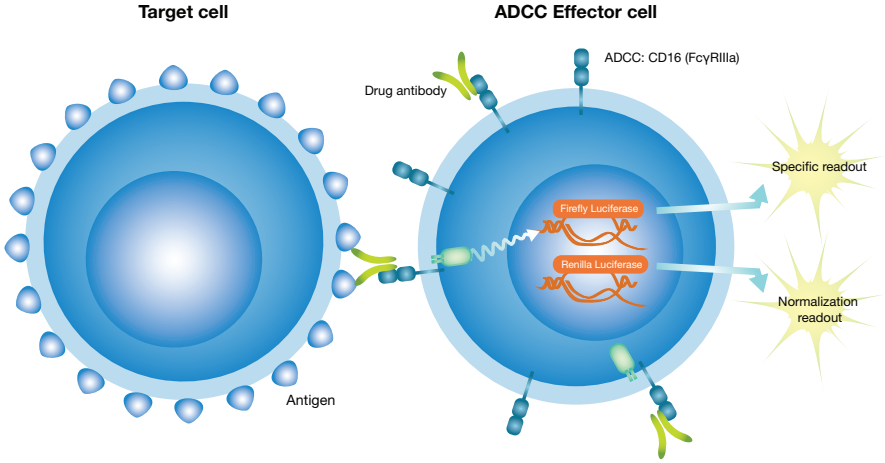


FIG. 1 - PRINCIPLE OF AN *ILITE* BIOASSAY

When a drug antibody binds to the antigen on the target cell, and the CD16 receptor of the effector cell binds to the Fc region of the antibody, several pathways are activated that induce a firefly luciferase reporter gene. The amount of luciferase generated can be used as a measure of ADCC activity. A Renilla luciferase gene is constitutively expressed in the effector cell for normalization purposes.

1. CELL LINE CHARACTERIZATION

BASIC ASSAY PERFORMANCE
AN EXAMPLE

As an important first step, we make sure that a clear dose-response curve can be generated from the assay when using increasing amounts of antibody in the presence of the target and effector cells. No firefly luciferase should be detected when using the negative control target cells. In addition, the Renilla luciferase should be detected at a constant level regardless of target cell and antibody concentration.

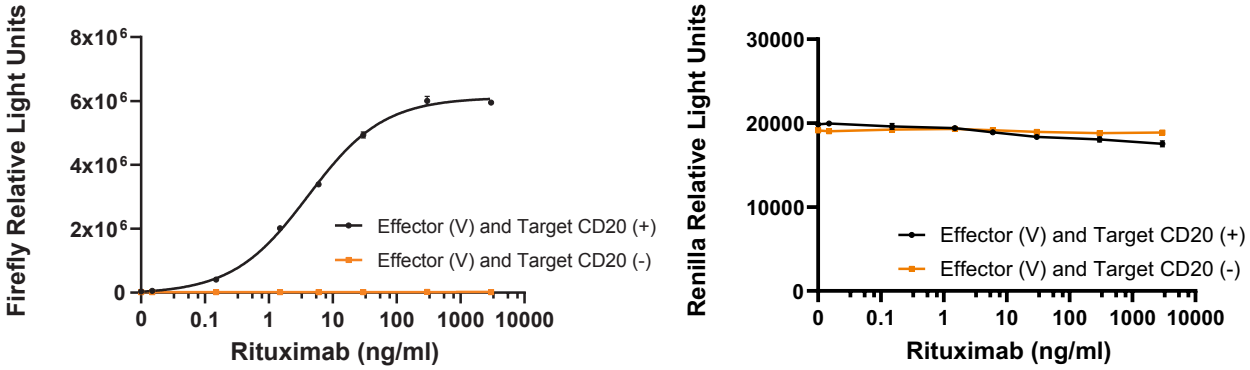
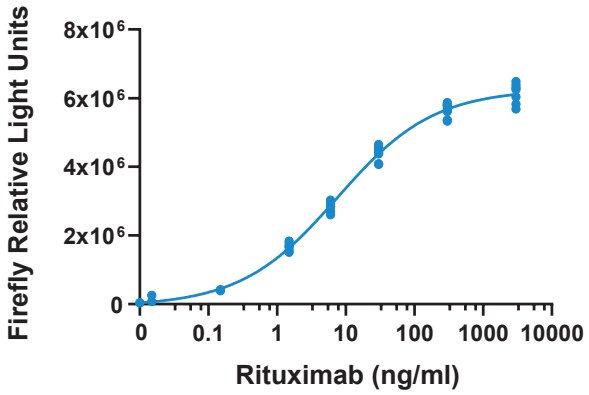


FIG. 2 - FIREFLY AND RENILLA LUCIFERASE READOUTS FROM AN *ILITE* ADCC ASSAY

Increasing amounts of rituximab were added in an iLite CD20 ADCC assay. A clear dose-response curve is observed when using CD20 positive target cells, whereas no signal is generated when using CD20 negative cells (Left). As expected, a constant signal is seen regardless of target cell type and rituximab concentration when measuring Renilla luciferase generated from the control gene (Right).

DYNAMIC RANGE
ASSAY APPLICATION EXAMPLE

When validating the cell lines, different potential applications are assessed using a standardized stimuli or commercial drug compound. Here, we present data on the 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).



Rituximab	In-assay (ng/ml)
A	3000
B	300
C	30
D	6.0
E	1.5
F	0.15
G	0.015
H	0

FIG. 3 - DYNAMIC RANGE.

Example of a CD20 ADCC assay where 0-3000 ng/ml rituximab was used in the assay to generate 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 and minimizes edge effects. 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.

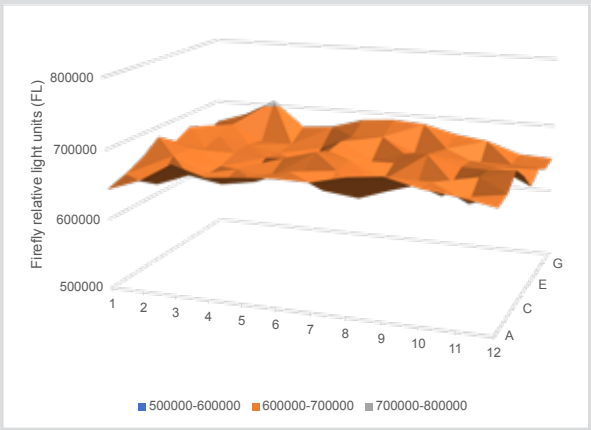


FIG. 4 - HOMOGENEITY

A single sample with 0.25 ng/ml rituximab 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.

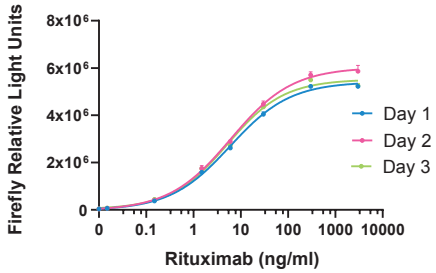
To assess the impact of any variance on the actual bioassays, key performance parameters such as:

- EC50
- Fold induction
- Hill slope

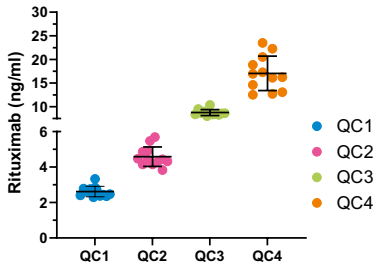
are determined using technical samples. All this is performed to establish the necessary information regarding how they perform in their designated assays/applications.

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	5.8	9
Fold induction	138	5
Hill Slope	0.69	4



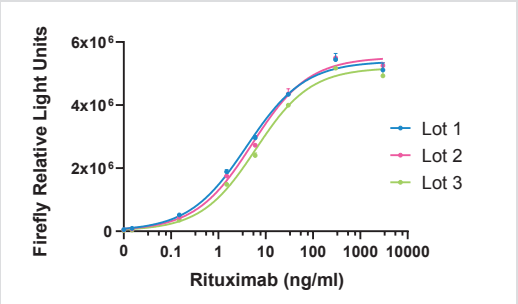
Sample ID	RIT (ng/mL)	CV%	Accuracy (%)
QC1	2.5	10	105
QC2	5.0	12	92
QC3	10	3	88
QC4	20	21	85

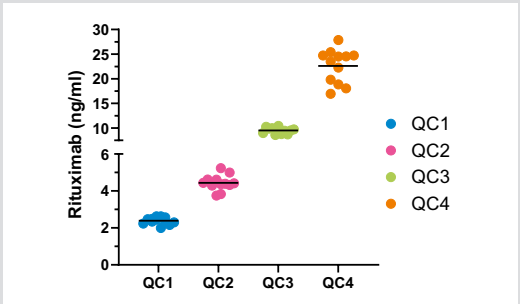
FIG. 5 - DAY-TO-DAY

The CD20 ADCC bioassay was performed with a dose-response curve and four technical samples with known amount of rituximab. Data shown is generated from an assay performed on three different days. RIT = rituximab

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.





Assay variable	Mean	CV%
EC50	5.1	21
Fold induction	158	45
Hill Slope	0.73	3

Sample ID	RIT (ng/mL)	CV%	Accuracy (%)
QC1	2.5	8	95
QC2	5.0	8	89
QC3	10	5	95
QC4	20	9	113

FIG. 6 - LOT-TO-LOT

The iLite CD20 ADCC bioassay was performed with a dose-response curve and four technical samples with known amount of rituximab. Data shown is generated from using three different batches of cells at the same assay occasion.
RIT = rituximab

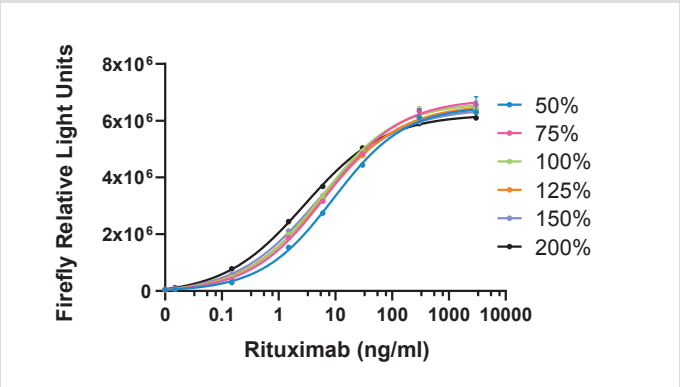
As shown in figure 6, the precision and accuracy of the technical samples are well within acceptable limits, with CVs generally below 10%, even though the absolute Firefly (FL) signal may vary. These results show that there are only small differences between assays performed on different days or using different batches, which is most probably due to the closely controlled production process and the assay ready format of the cells.

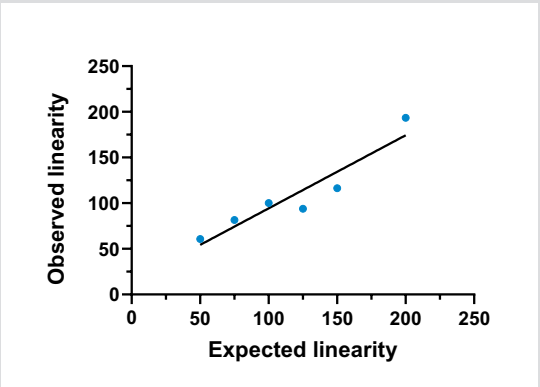
LINEARITY (PARALLELISM) FUNCTIONAL / LINEARITY (PARALLELISM) INHIBITORY

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.





Expected linearity	50%	75%	100%	125%	150%	200%
EC50	9.2	6.8	5.6	5.9	4.8	2.9
Accuracy	82%	92%	N/A	133%	129%	103%
Hill Slope	0.68	0.65	0.66	0.62	0.61	0.61
Fold induction	130	127	131	137	133	138

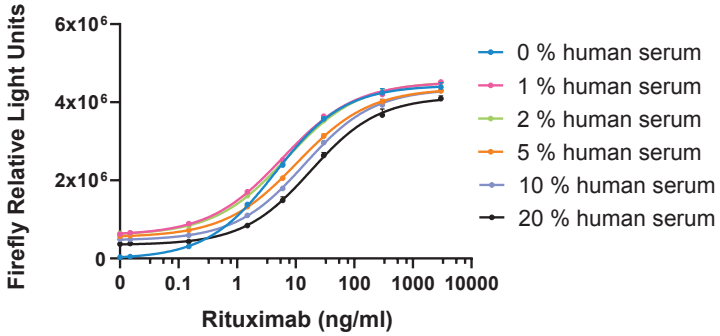
FIG. 7 - PARALLELISM / LINEARITY

The iLite CD20 ADCC 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.

3. CELL LINE BIOASSAY PRE-CLINICAL & CLINICAL APPLICATIONS

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 conc.	0%	1%	2%	5%	10%	20%
Hillslope	0.74	0.69	0.70	0.70	0.73	0.75
EC50	4.5	5.8	7.0	11	14	18
Fold induction	130	6.7	6.6	7.1	8.0	10

FIG. 8 - SERUM TOLERANCE

The iLite CD20 ADCC bioassay was performed with a dose-response curve including human serum, up to 20%. As seen in the figure, the presence of serum is well tolerated in the assay.

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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