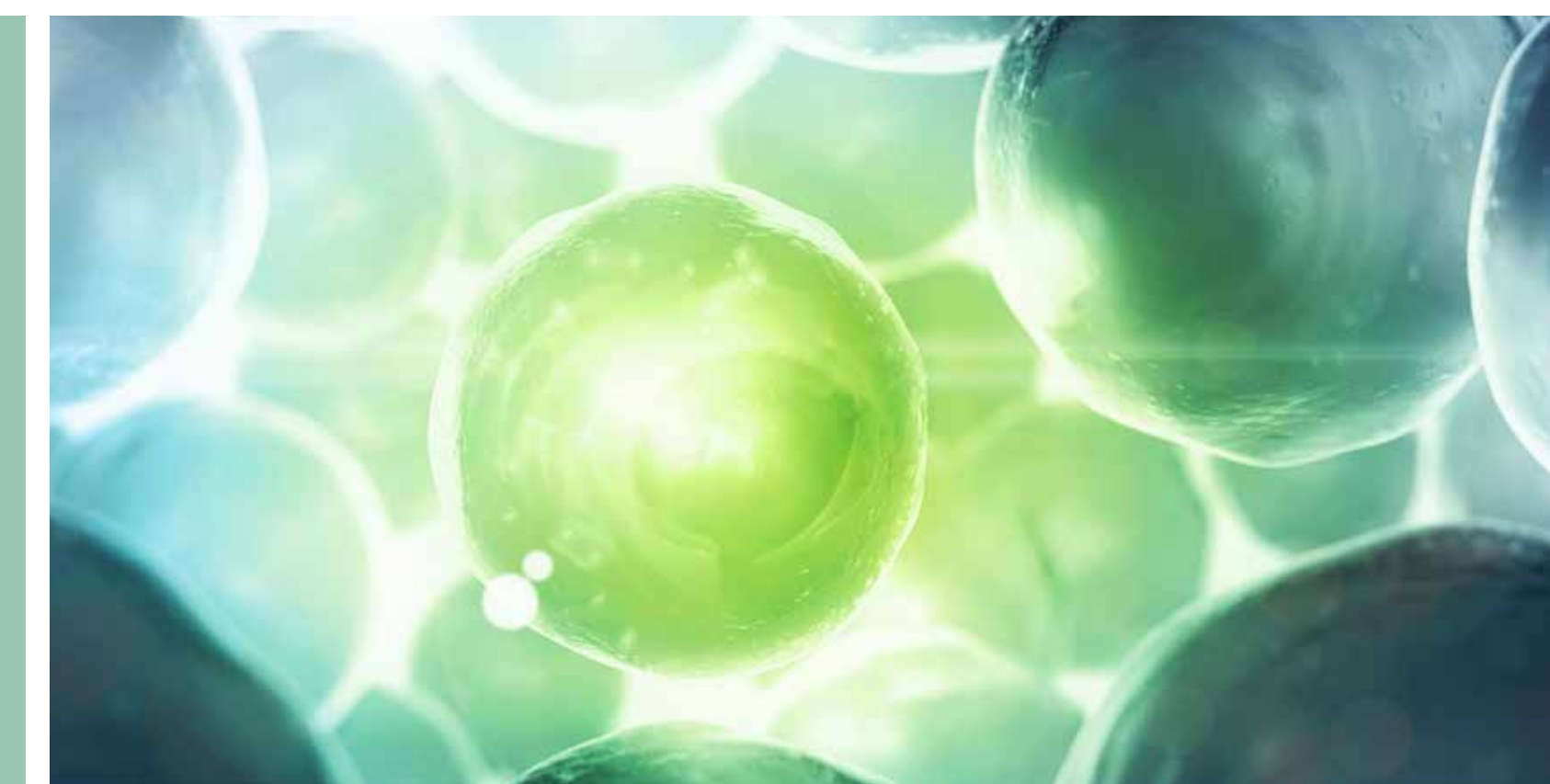


Performance Characteristics of *iLite*[®] Cell-based Reporter Gene Assays: Case Study of *iLite*[®] IL-23 Assay Ready Cells

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Introduction

Biological activity is an essential quality attribute for the assessment of the potency and stability of biological drugs, and the successful development of such biologics is dependent upon the establishment of validated and standardized assays that allow direct comparisons of the relative potency and stability of different batches.

We have developed a high throughput assay platform, *iLite*[®], based on the use of engineered reporter-gene cell lines that is applicable to most biologics and that allows the direct comparison of drug potency and stability in the same assay. Furthermore, since biological activity is measured, the same assay system can be used to determine the presence of neutralizing antibodies.

Here, using our *iLite*[®] IL-23 Assay Ready Cells as an example, we show the robustness of our assay system, describing parameters such as accuracy, precision, and plate homogeneity, as well as assay characteristics such as EC50 and LLOQ.

Cell Line Development

The IL-23 reporter gene assay was established using the avian B-cell line DT-40 that does not require IL-23 or other human cytokines in order to proliferate and that is unresponsive to the growth factors present in human serum. Thus, DT-40 cells were co-transfected with the IL-23R and IL-12Rb1 receptor chains together with a STAT5 expression vector, a STAT5 responsive Firefly luciferase (FL) reporter gene construct, and the Renilla luciferase (RL) normalization gene under the control of a constitutive promoter.

Methods

Assays ready IL-23 cells were thawed and diluted in RPMI + 9% HI-FBS +1% P/S. IL-23 protein (R&D Systems) was diluted in same buffer and both incubated for 5h at 37°C/5% CO₂. Detection of Firefly and Renilla Luciferase activity was determined with Dual-Glo[®] Luciferase Assay System (Promega) and read sequentially. Standard dose response curves were run with IL-23 protein 0-63 ng/ml, 3-fold dilution steps (63, 21, 6.9, 2.3, 0.77, 0.26, 0.086 and 0 ng/ml). Samples of IL-23 protein, Q1, Q2 and Q3 were diluted to 2.0 ng/ml, 1.5 ng/ml and 1.0 ng/ml. Assay plate homogeneity (FL RLU) was explored using a solution of 0.5 ng/ml IL-23 protein in all 96 wells of a plate.

Results

The robustness of the *iLite*[®] IL-23 Assay Ready Cells was explored by testing a number of different characteristics and parameters including repeatability, intermediate precision, accuracy and plate homogeneity. The results show that the assay has high repeatability and precision as well as good accuracy. In addition, the assay gives a high fold induction (19x) as well as a lower limit of quantification (LLOQ) in the low nanoliter range (0.1 ng/ml). Taken together, the results show that the *iLite*[®] Technology and the Assay Ready format is capable of generating a high performing bioassay, suitable for many applications within the drug continuum.

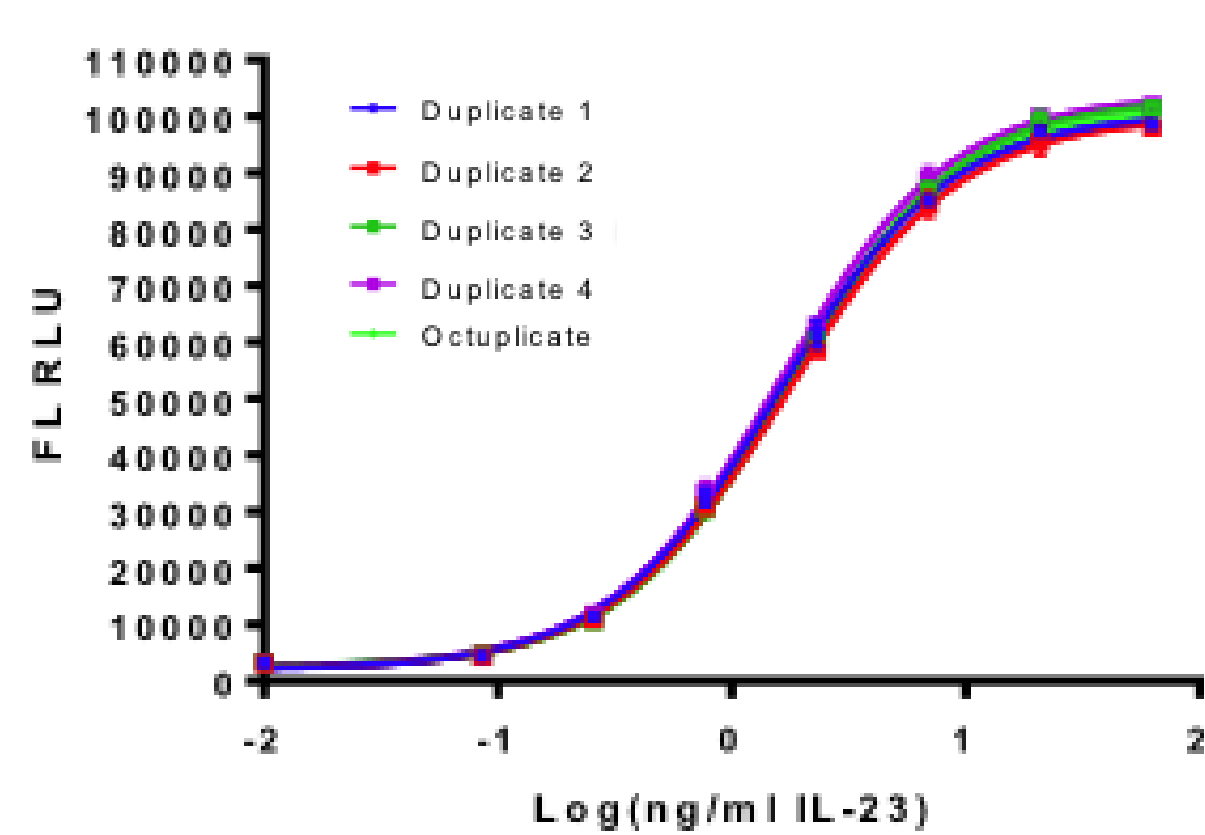
Assay characteristics

Dose response curve	0-63 ng/ml, 3-fold dilution
EC50†	1,40 (1,10-1,72)
Fold induction†	19 (15-22)
LLOQ*	0.1 ng/ml
ULOQ*	5.1 ng/ml

* LLOQ, ULOQ – defined as lowest and highest concentration of analyte which can be quantified in this assay set-up with 75-125% accuracy and CV<25% precision.

† EC50 and fold-induction - average from 20 dose response curves, tests at separate days and with different cell batches (90% CI)

Repeatability (Within run)



Firefly RLU Results within-run (1 plate)						Precision
	Duplicate 1	Duplicate 2	Duplicate 3	Duplicate 4	Octuplicate	CV%
Best-fit values						
BOTTOM	2079	2208	2473	2325	2270	7%
TOP	100565	99928	103095	103757	101843	2%
HILLSLOPE	1,18	1,17	1,21	1,19	1,19	1%
EC50	1,60	1,72	1,77	1,61	1,67	5%
Goodness of Fit						
R ²	1,00	1,00	1,00	1,00	1,00	0%
Fold Induction 21/0.086 ng/ml	22	22	22	21	22	1%

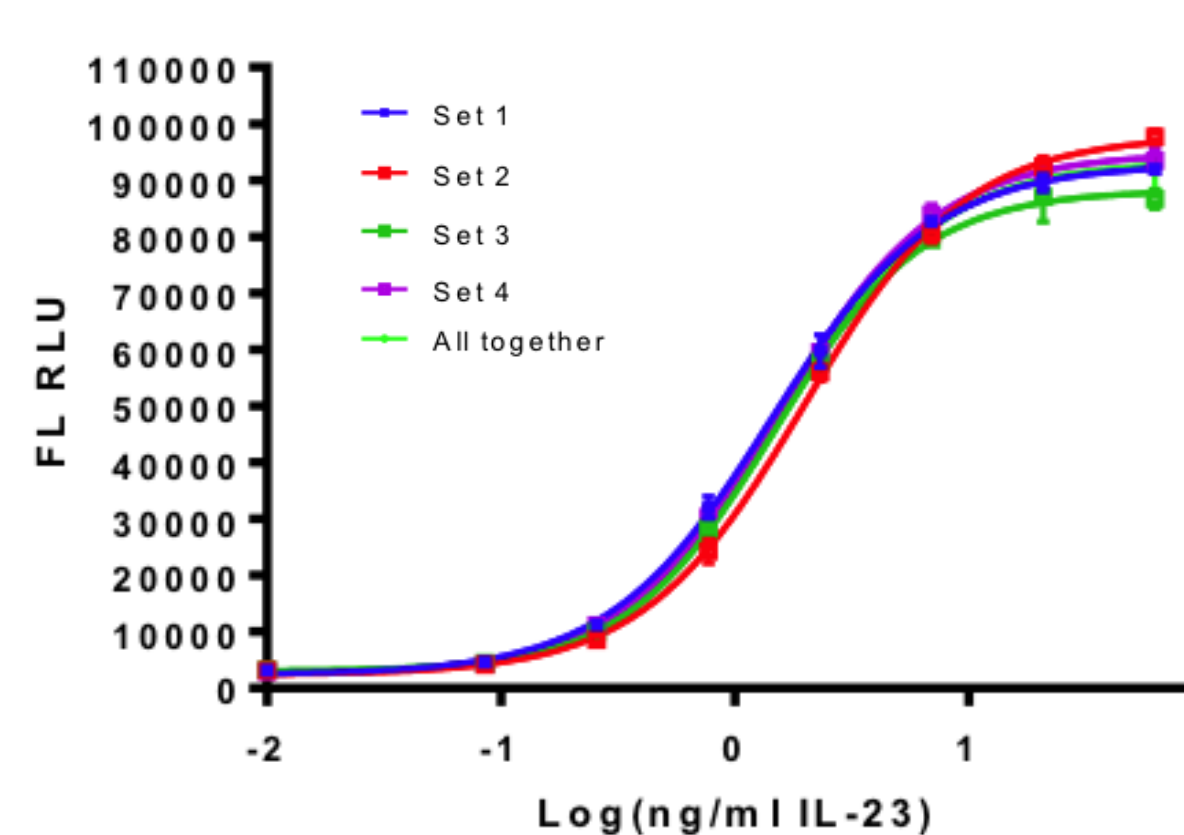
Four sets of dose-response curve in duplicates, Samples in eight wells.

Sample Accuracy ¹ (ng/ml ²)									
	FLU	Curve 1	Curve 2	Curve 3	Curve 4	Octuplicate	Avr	CV%	Acc%
QC1	54441	1,78	1,94	1,87	1,68	1,81	1,82	7%	91%
QC2	50130	1,53	1,67	1,62	1,46	1,57	1,57	5%	105%
QC3	35479	0,908	0,981	0,977	0,876	0,934	0,94	5%	94%

¹Firefly RLU Results between-runs (4 plates)

²IL-23 protein diluted to 2.0 ng/ml, 1.5 ng/ml and 1.0 ng/ml

Repeatability (Between runs)



Firefly RLU Results between-runs (4 plates)						Precision
	Plate 1	Plate 2	Plate 3	Plate 4	CV%	
Best-fit values						
BOTTOM	2573	2822	2626	2748	4%	
TOP	70661	68969	70921	68494	2%	
HILLSLOPE	1,31	1,37	1,30	1,36	3%	
EC50	1,27	1,20	1,28	1,21	3%	
Goodness of Fit						
R ²	1,00	1,00	1,00	1,00	0%	
Fold Induction 21/0.086 ng/ml	17	16	16	16	2%	

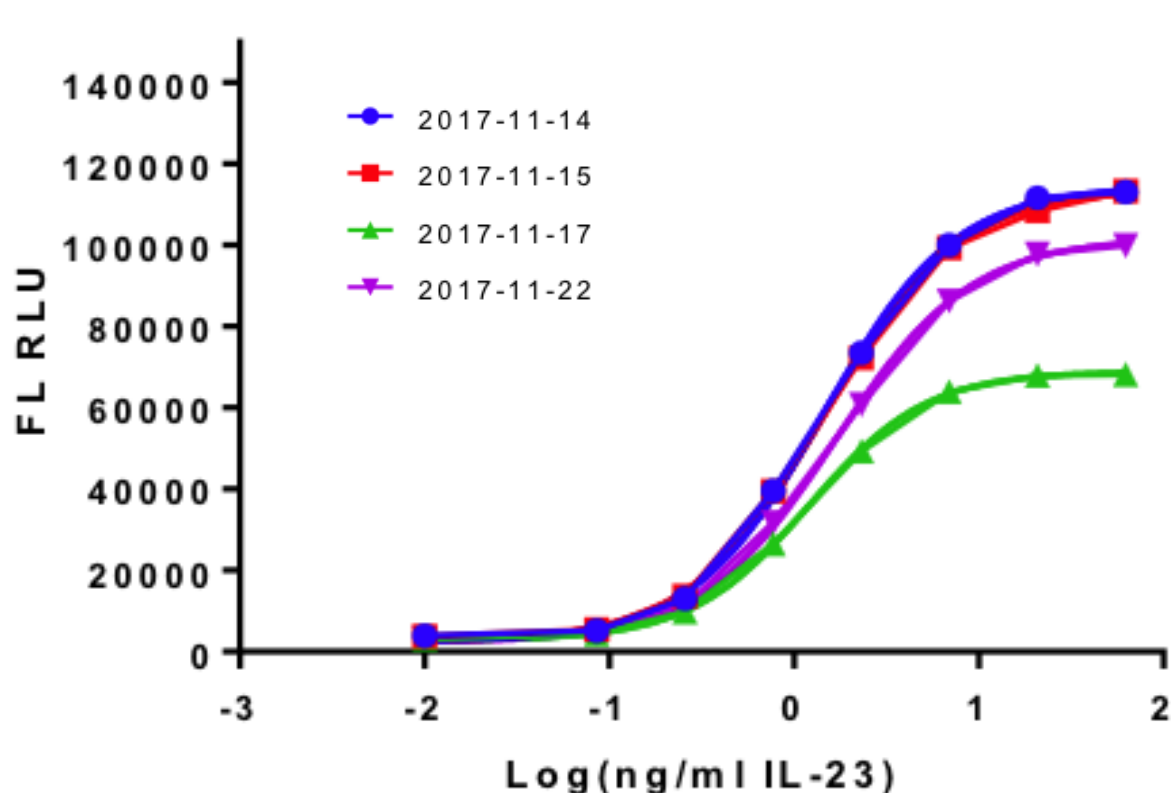
One set of dose response curve in duplicates, and each sample in quadruplicate. Four plates. All same day.

Sample Accuracy ¹ (ng/ml ²)											
	Plate 1	Plate 2	Plate 3	Plate 4	Plate 1	Plate 2	Plate 3	Plate 4	Avr	CV%	Acc%
QC1	45938	45010	45138	44365	1,95	1,82	1,88	1,81	1,86	3%	93%
QC2	43563	42783	43415	43945	1,74	1,64	1,73	1,77	1,72	3%	115%
QC3	33498	32278	31475	31745	1,10	1,02	1,01	1,02	1,04	4%	104%

¹Firefly RLU Results between-runs (4 plates)

²IL-23 protein diluted to 2.0 ng/ml, 1.5 ng/ml and 1.0 ng/ml

Intermediate precision (Between Days)



Firefly RLU Results Between Days						Precision
	Day 1	Day 2	Day 3	Day 4	CV%	
Best-fit values						
BOTTOM	2503	2418	2622	2270	7%	
TOP	114790	114007	68969	101843	22%	
HILLSLOPE	1,23	1,19	1,37	1,19	6%	
EC50	1,45	1,46	1,20	1,67	9%	
Goodness of Fit						
R ²	1,00	1,00	1,00	1,00	0%	
Fold Induction 21/0.086 ng/ml	21	20	16	22	5%	

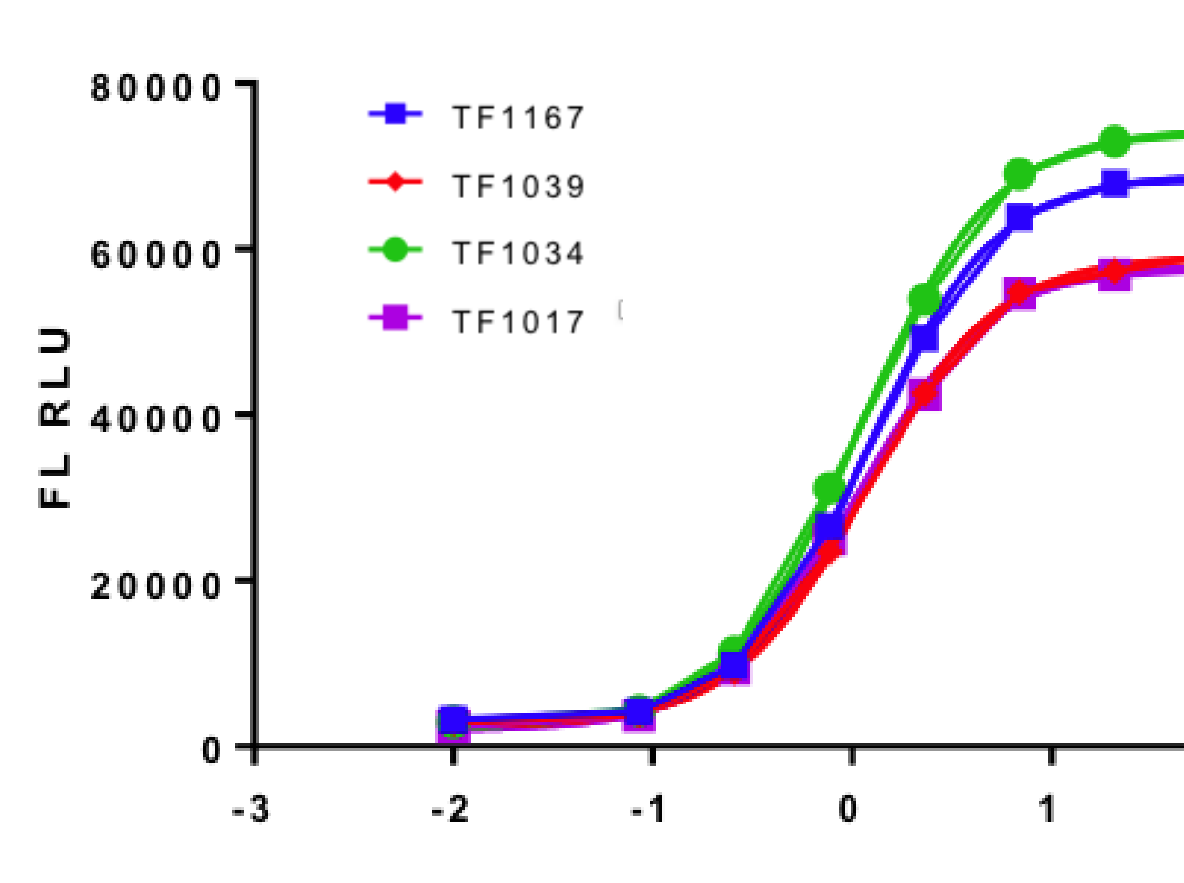
One set of dose response curve in duplicates, and each sample in quadruplicate. Repeated four days.

Sample Accuracy ¹ (ng/ml ²)											
	Plate 1	Plate 2	Plate 3	Plate 4	Plate 1	Plate 2	Plate 3	Plate 4	Avr	CV%	Acc%
QC1	67830	64590	45010	54441	1,90	1,78	1,82	1,81	1,86	3%	93%
QC2	58103	58355	42783	50130	1,43	1,47	1,64	1,57	1,54	6%	102%
QC3	42105	43350	32278	35479	0,884	0,922	1,02	0,934	0,97	6%	97%

¹Firefly RLU Results between-runs (4 plates)

²IL-23 protein diluted to 2.0 ng/ml, 1.5 ng/ml and 1.0 ng/ml

Between Assay Ready Cell batches



Firefly RLU Results between cell batches					Precision
	TF1017	TF1034	TF1039	TF1167	CV%
Best-fit values					
BOTTOM	2822	2488	2267	1999	15%
TOP	68969	59181	74523	58170	12%
HILLSLOPE	1,37	1,33	1,30	1,31	2%
EC50	1,20	1,17	1,09	1,06	6%
Goodness of Fit					
R ²	1,000	0,999	0,999	0,999	0%
Fold Induction 21/0.086 ng/ml	16	15	17	15	5%

One set of dose response curve in duplicates, and each sample in quadruplicate. Four cell batches.

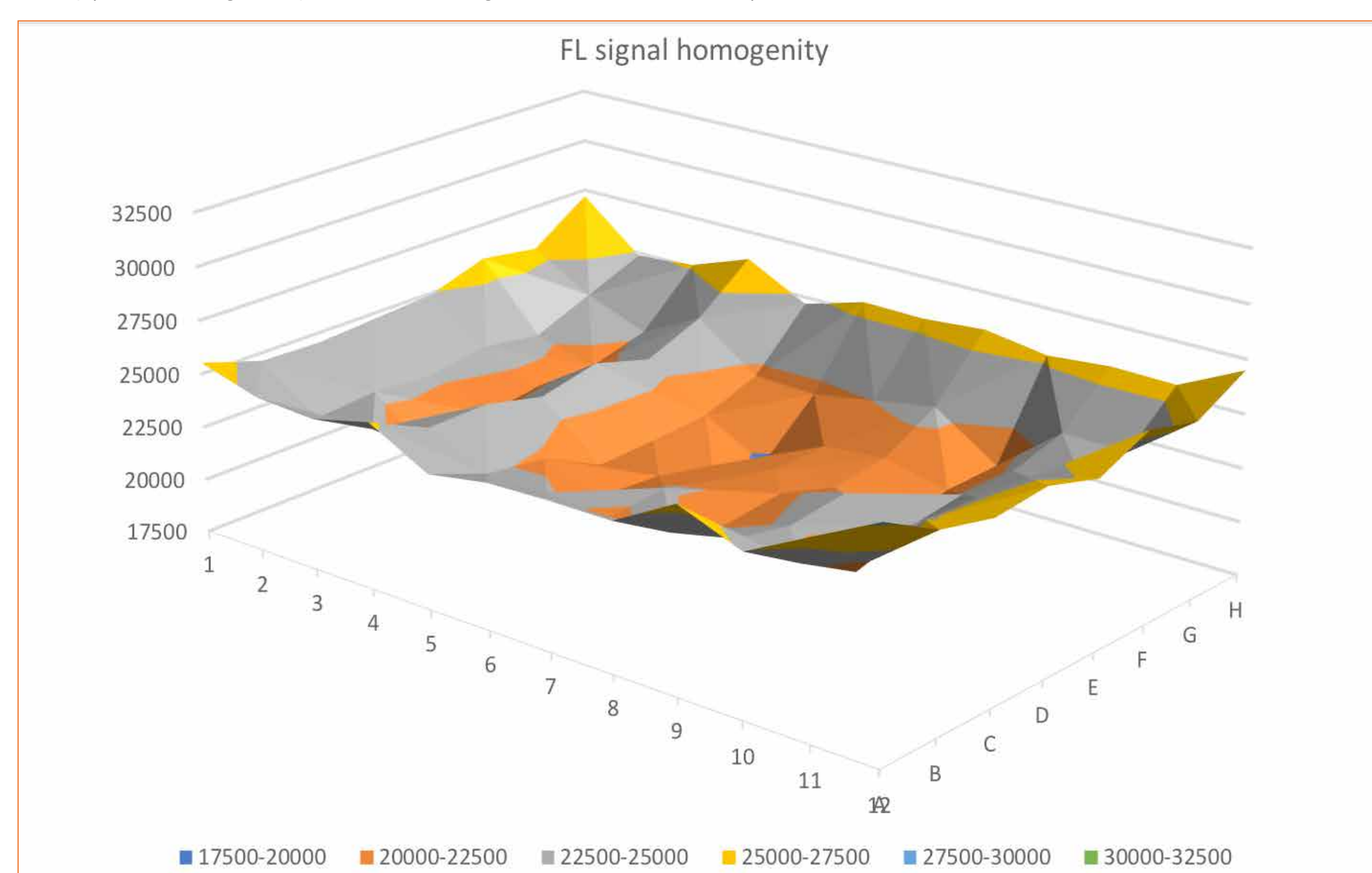
Sample Accuracy ¹ (ng/ml ²)											
	TF1017	TF1034	TF1039	TF1167	TF1017	TF1034	TF1039	TF1167	Avr	CV%	Acc%
QC1	45010	39763	50893	37688	1,82	1,90	1,90	1,61	1,81	8%	90%
QC2	42783	39100	48408	39783	1,64	1,83	1,69	1,83	1,75	6%	117%
QC3	32278	24978	33255	30290	1,02	0,853	0,876	1,07	0,95	11%	95%

¹Firefly RLU Results between-runs (4 plates)

²IL-23 protein diluted to 2.0 ng/ml, 1.5 ng/ml and 1.0 ng/ml

Plate Homogeneity

Assay plate homogeneity (FL RLU): 0.5 ng/ml in all 96 wells of a plate.



Summary and Conclusions

By using the *iLite*[®] IL-23 Assay Ready Cells as an example, we have shown the robustness of our assay system, describing parameters such as accuracy, precision, and plate homogeneity, as well as assay characteristics such as EC50 and LLOQ. The results demonstrate that using this system, assay parameters normally associated with ligand binding assays can be achieved also with a cell-based setup.

Accuracy

	Expected value	Measured value	Accuracy
Sample 1	2.0	1.83 (1.68-1.95)	92% (84-98%)
Sample 2	1.5	1.61 (1.46-1.83)	107% (97-122%)
Sample 3	1.0	0.98 (0.88-1.07)	98% (88-107%)

Average from 20 duplicates, tests at separate days and with different cell batches (90% CI)

Assay plate

FL RLU homogeneity (CV%)	
Row	<5%
Column	<9%
Plate	8%

Precision (CV%)

		EC50	Fold induction	Sample 1	Sample 2	Sample 3
Repeatability	Within run (Octuplicates)	5%	1%	7%	5%	5%
	Between Runs (4 plates)	3%	2%	3%	3%	4%
Intermediate precision	Between Days (4 days)	9%	12%	3%	6%	6%
	Between IARC batches (4 batches)	6%	5%	8%	6%	11%