Biacore™ Insight Evaluation Software for PLA and EC50 potency analysis

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Introduction

Potency assays are used throughout the development of biotherapeutics to measure the biological activity of candidates, either directly using functional assays or indirectly using surrogate potency assays such as Biacore surface plasmon resonance (SPR)-based assays.

In Biacore systems, potency is assessed relative to a reference sample and typically relies on comparing dose-response curves for a drug candidate and reference, respectively, against an immobilized or captured target molecule.

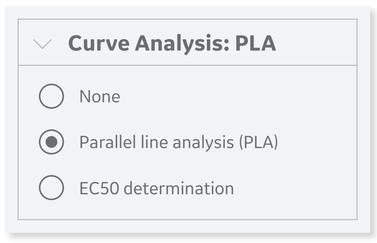
Parallel line analysis (PLA) and EC50 calculations are often used to quantitate similarities of the dose-response curves. So far, PLA and EC50 analysis of Biacore data have been handled using external software such as Microsoft® Excel®.

This poster presents new features in Biacore Insight Evaluation Software enabling PLA and EC50 curve analysis. The data was generated in Biacore 8K or Biacore 8K+ as well as Biacore T200. In Biacore 8K and Biacore 8K+, full GxP support is offered for runs and evaluations.

Biacore Insight Evaluation Software

- A software with modular design for Biacore 8K and Biacore 8K+ systems
- Supports evaluation of imported Biacore T200 and Biacore S200 data
- Full GxP support for PLA/EC50 runs performed in Biacore 8K Control software 2.0
- Application-specific evaluation software packages allow a more cost-efficient configuration, which expands as your screening and characterization requirements evolve

PLA and EC50 determinations are included in the Concentration and Potency Extension package



References

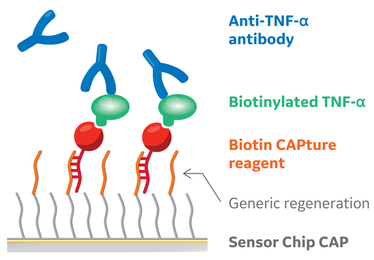
1. High-resolution SPR-based surrogate potency assay to facilitate comparability and potency assessment. GE Healthcare poster, KA1323071117PO (2017).
2. Frostell *et al.* Surrogate potency assays: Comparison of binding profiles complements dose response curves for unambiguous assessment of relative potencies. *J. Pharm. Analysis*, **8**, 138–146 (2018).

Application example

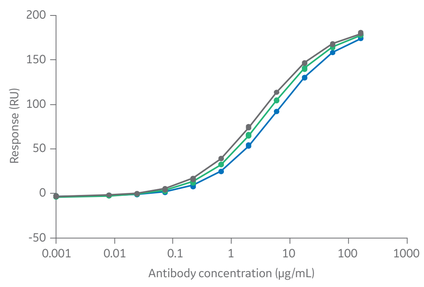
Surrogate potency assay using Biacore systems

We previously presented how Biacore T200 may be used to set up surrogate potency assays to facilitate comparability and biosimilar studies (1, 2). Following a linearity test of anti-TNFα antibodies with 70% to 130% nominal concentrations, data were evaluated using Excel.

(A)



(B)



(C)

Sample relative conc. (%)	EC50 (µg/mL)	Relative potency (%)
100 (reference)	4.23	100.0
130	3.30	128.1
70	5.90	71.7

(A) A capture-based format, minimizing assay development time, was demonstrated for relative potency measurements. The example shown uses biotinylated TNF-α and a concentration series of anti-TNF-α antibodies. (B) Linearity test to prove the assay was fit for purpose. (C) Relative potencies as calculated in Excel.

PLA and EC50 analysis of Biacore T200 data

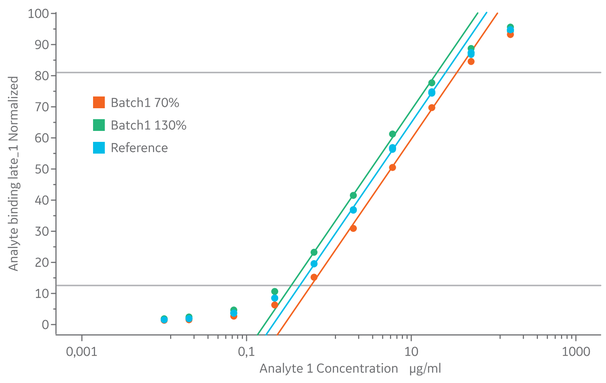
Following import of Biacore T200 data (blr-file), the data was quickly re-evaluated using the built-in EC50 and PLA curve analysis functions in Biacore Insight Evaluation Software, resulting in good agreement with the relative potencies determined above.

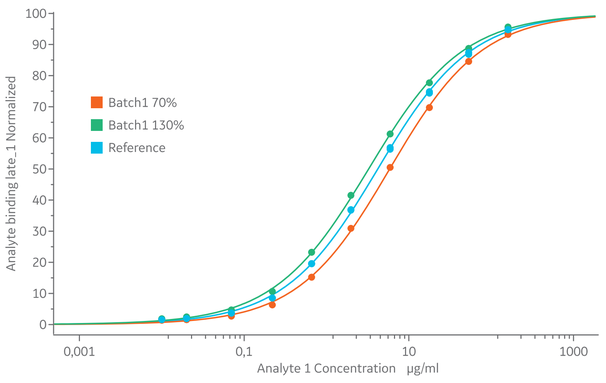
PLA analysis

- Linear fit to the linear part of the response vs logarithmic concentration assuming a common slope

EC50 analysis

- Four-parameter equation fitted to response vs concentration
- Restricted fit option helps to compare between results (enables global fit of asymptotes and Hill coefficient)





Plot table **PLA results** PLA settings

Group	Control	Sample	Relative potency	95% confidence low	high	Common slope	Common R2	Control slope	Sample slope	Control R2	Sample R2	Slope ratio S/C
Ch 1	Reference	Batch1 70%	72,89	68,03	78,07	38,68	0,9985	38,77	38,41	0,9993	0,9976	0,9908
Ch 1	Reference	Batch1 130%	127,1	120,6	134,0	38,68	0,9991	38,77	38,41	0,9993	0,9988	0,9907

Plot table **EC50 results** EC50 settings and parameters

Group	Control	Sample	Relative potency	95% confidence low	high
Ch 1	Reference	Batch1 70%	71,64	65,54	78,30
Ch 1	Reference	Batch1 130%	129,1	119,3	139,7

Plot table EC50 results **EC50 settings and parameters**

Group	Curve	EC50	Rlo	Rhi	A2	Chi2	Function
Ch 1	Reference	4,194	0,01204	100,0	0,7647	0,3676	4 param
Ch 1	Batch1 70%	5,854	-2,170e-3	100,0	0,7757	0,4897	4 param
Ch 1	Batch1 130%	3,249	0,02849	100,0	0,7553	0,5454	4 param

The results table include 95% confidence interval for PLA and EC50, which statistically defines with 95% probability that the relative potency is within calculated low and high limits. Slope parameters for PLA monitor how valid the common slope assumption is.

Conclusions

- Biacore Insight Evaluation Software has dedicated software tools, available in the Concentration and Potency Extension package, for PLA and EC50 determinations.
- Surrogate potency assays performed in Biacore 8K, Biacore 8K+, or Biacore T200 can easily be evaluated without the need to export to Excel or similar software.
- The re-evaluation of a previous Biacore T200 study showed that the results can be reproduced in a much easier way with higher data integrity.

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