Biacore application guides

Potency measurements with Biacore systems

cytiva

Potency measurements represent one of several tests used in development and manufacturing of biotherapeutics to quantify the relevant therapeutic activity or intended biological effect. Bioassays such as animal studies or cell culture systems may determine potency directly by measuring the actual biological activity. In contrast, binding assays such as ELISA and SPR-based techniques measure binding of the therapeutic compound to a selected target molecule, and are often referred to as surrogate potency assays. The relevance of surrogate assays to true potency measurements depends on how well the binding correlates with the biological effect.

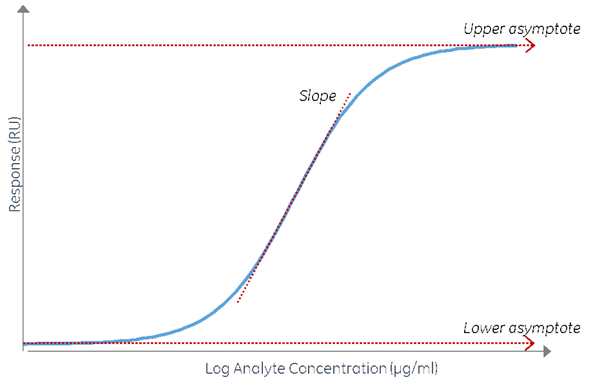
Potency in Biacore systems

In Biacore™ systems, potency is assessed relative to a reference substance, measured in the same assay run as the test substance, by comparison of dose-response curves (plots of response against log (concentration) for reference and test substances. Although measurements from separate runs can be evaluated together, the effects of run-to-run variation then need to be considered.

Potency measurements are currently supported only in Biacore Insight Evaluation Software, which allows evaluation of results from Biacore T200, Biacore 8K and Biacore 8K+ systems. Two calculations can be used:

- Parallel line analysis (PLA), which compares the horizontal position of dose-response curves for test and reference substances (see PLA analysis background, on page 10)
- Half-maximal effective concentration (EC_{50}) which determines the concentration of substance required to achieve half-maximal binding response (see EC_{50} analysis background, on page 11)

The dose response curve with a logarithmic concentration scale is generally sigmoid, with upper and lower asymptotes and a more or less linear central region. Evaluation by PLA is based on the slope of the linear region. Evaluation in terms of EC_{50} relies on determination of the upper and lower asymptotes by fitting a four-parameter function to the experimental data.



Terminology

Term	Meaning
Potency	Quantitative measure of therapeutic activity of a biopharmaceutical substance.
Surrogate potency	Potency determined from an in vitro binding assay.
Relative potency	Potency of a test sample relative to that of a reference substance.
PLA	**Parallel Line Analysis** (sometimes called **Parallel Line Assay**), a method for determining relative potency by comparing dose-response curves for test and reference substances.
EC_{50}	Half-maximal effective concentration, the concentration that gives 50% of the maximum effect. Relative potency can be determined by comparing EC_{50} values for test and reference substances.
Dose-response curve	In the context of Biacore systems, a plot of binding response against analyte concentration. Concentration is usually plotted on a logarithmic scale.

Tips for potency measurements

- Use relatively high ligand immobilization levels to ensure confidently measurable responses from low analyte concentrations. Optimal ligand levels are determined as part of assay development.
- Make sure that sufficient concentrations of each reference and test substance are used to establish the dose-response curve adequately. The recommended minimum is 9 concentrations evenly distributed over the lower asymptote, central, and upper asymptote regions of the curve. Establish suitable concentration ranges during assay development or in pilot experiments.
- For PLA evaluation, there should be at least three (preferably more) points on the linear region of the dose-response curve, for both reference and test substances. Upper and lower asymptotes are required for establishing the dose-response curve during assay development, but for established assays only the linear region needs to be covered.
- For EC_{50} evaluation, the concentration range should be sufficiently wide for confident determination of the upper and lower asymptotes of the dose-response curve
- Prepare concentration series of reference and test substances using identical buffers (dilution in runnng buffer is recommended)
- Place report point for binding level measurement shortly after the end of the sample injection, to avoid bulk refractive index contributions. If this is not appropriate (for example, if the analyte dissociates rapidly from the ligand), match the buffer composition of samples and running buffer as closely as possible.
- Substances defined as positive controls in the Insight Evaluation Software are automatically treated as reference substances

General Considerations

Assay format

Potency measurements are always based on direct binding assays and evaluated in terms of dose-response curves. The ligand (target molecule) may be immobilized or captured on the sensor surface. If ligand is captured, it is important that the capturing level is constant over the whole assay. Enhancement assays are not recommended for potency measurements.

Assay development

In addition to the general assay development requirements for assays with Biacore systems such as establishment of immobilization and regeneration conditions, development of potency assays involves determination of suitable immobilization level and analyte concentration range for full coverage of the dose-response curve.

- Develop the assay using reference samples or equivalent. Some adjustment may be necessary for test samples when the assay is run, but the same concentration range is usually appropriate for both test and reference samples unless the potencies are widely different.
- Establish full dose-response curves during assay development regardless of whether the assay will be evaluated using PLA or EC_{50}. As a general recommendation use 9 concentrations of analyte. Aim for three points on each of the lower asymptote, central, and upper asymptote regions for complete determination of the dose-response curve. The concentration range can be restricted to the linear region for PLA when the assay is run: however, EC_{50} evaluation requires determination of the upper and lower asymptotes.
- Bear in mind that at least three points on the linear region of the dose-response curve are recommended for evaluation by PLA. EC_{50} determination requires sufficient (recommended at least 9) points on the dose-response curve to determine the complete curve reliably.

Surface preparation

Amount of immobilized ligand

Follow the guidelines below with respect to the amount of immobilized ligand.

- Use relatively high immobilization levels to enable measurements at lower analyte concentrations and allow confident determination of the lower asymptote of the dose-response curve
- Avoid excessively high immobilization levels that may consume unnecessarily large amounts of analyte and create difficulties in reaching the upper asymptote of the dose-response curve
- If ligand is attached to the surface using a capturing approach, it is important that the capture level is consistent between cycles

Optimal ligand levels should be determined as part of assay development. The ligand level should be sufficient to give confident measurement of low analyte concentrations, to establish the lower asymptote of the dose-response curve. A suitable starting level for immobilization of a ligand with molecular weight about 50 000 on Sensor Chip CM5 is usually in the range 2500 to 4000 RU. Small analytes will generally require higher ligand levels.

Reference surface

A reference surface can be used to confirm that the samples do not bind to the dextran matrix on the sensor chip. Any non-specific binding to the dextran matrix should be eliminated or minimized during assay development.

Reference subtraction, however, is not normally used for potency measurements.

Sample preparation

Since relative potency measurement relies on comparison between dose-response curves, it is important that the test and reference samples are prepared as far as possible in the same way. Bear the following points in mind with respect to sample preparation:

- Match samples and running buffer as closely as possible with respect to refractive index. Prepare sample dilutions in running buffer.
- Use a dilution series so that there are 3 or more points on the linear region of each dose-response curve for PLA and 9 points covering the full range from lower to upper asymptote for EC_{50} determination
- Prepare reference and test samples in the same way
- Where possible, determine the stock solution concentrations using the same method for reference and test substances. The dose response curve for potency measurement reflects the amount of analyte that can bind to the ligand, which may not be the same as the concentration determined by other techniques. Ideally, determine the concentration using a Biacore-based assay.

Experimental setup

Workflow

A general workflow for potency analysis is summarized below. Adjust the workflow as required for your application.

Step	Action
1	Prepare the sensor surface by immobilizing ligand or capturing molecule.
2	Run 1 to 10 startup cycles to stabilize the surface. Recommended startup cycles include analyte at one selected concentration and regeneration.
3	Run test and reference samples in a single assay step to accommodate the recommended run order (see *Sample run order*, on page 10).
4	For runs performed in Biacore 8K and Biacore 8K+ systems, set reference substances as positive controls in the run method or at the start of evaluation (see Evaluation workflow, on page 13). For runs performed in other Biacore systems, set reference substances as positive controls at the start of evaluation.
5	Run concentration series for reference and test substances from low to high concentrations. See *Sample run order,* on page 10 for considerations of sample run order.

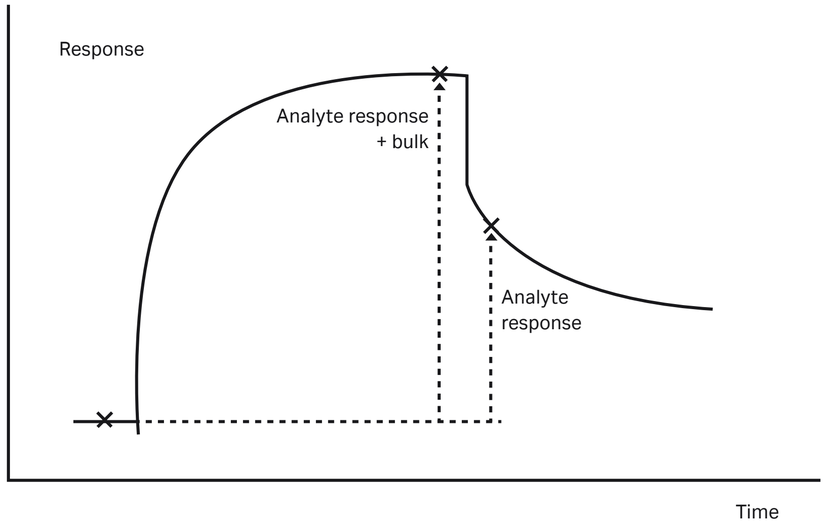
Parameters and settings

The table below summarizes recommended settings.

Parameter	Recommendation	Comments
Flow rate for capture	10 µL/min	Minimize ligand consumption.
Contact time for capture	60 to 180 s	According to assay requirements.
Flow rate for sample	10 µL/min	Minimize sample consumption.
Contact time for sample	60 to 400 s	Dependent on binding rate. Longer contact times can increase assay sensitivity.
Dissociation time for sample	0	Dissociation time is not relevant for potency measurements.
Flow rate for regeneration	30 µL/min	Use the same flow rate as the sample injection if the response stabilizes slowly after regeneration.
Contact time for regeneration	30 to 180 s	Depending on assay requirements.

Report point placing

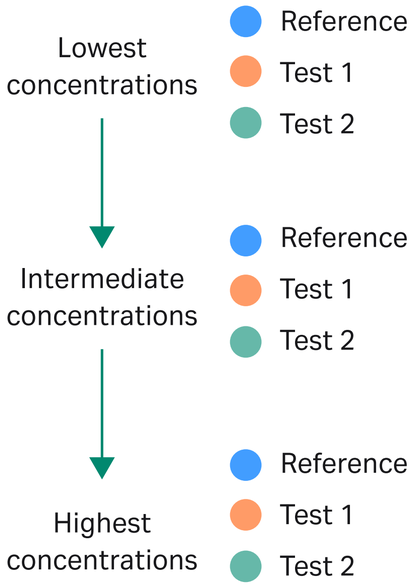
Report points for potency analysis should preferably be placed shortly (5 to 30 s) after the end of the sample injection, to avoid bulk refractive index contributions. If analyte dissociation is rapid, it may be necessary to place the report point before the end of the injection. In such cases, careful matching of the bulk refractive index in samples and running buffer to minimize bulk contributions becomes more important.



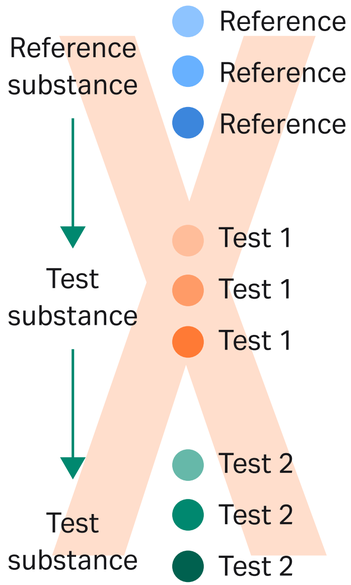
Make sure that report point placing is the same for reference and test substances.

Sample run order

Potency assays involve determination of multiple dose-response curves, often requiring many analysis cycles and long run times. To minimize the effect of assay drift during the run, group the run order by sample concentration (recommended low to high) regardless of sample name. With the more intuitive arrangement where the concentration series for each sample is kept together, there will be a greater potential impact of assay drift on comparison between curves for different substances.



Group the sample run order by concentration (low to high) regardless of sample name.



Do not group the sample run order by sample name, even if this is more intuitive.

Regeneration

Efficient regeneration of the surface between cycles is a requirement for reliable potency determinations. Optimal regeneration conditions must be addressed during assay development. Incomplete regeneration or loss of the binding activity from the surface will impair the performance of the assay and the useful lifetime of the sensor chip may be shortened.

Evaluation

PLA analysis background

PLA stands for Parallel Line Analysis and gives the user a statistical mean to compare two or more curves quantitatively. PLA calculates relative potency results by comparing the response generated by the test sample versus that generated by the reference substance over the linear region of the dose-response curve. PLA also generates statistical values that measure the parallelism between reference and sample dose-response curve.

PLA estimates relative potency by fitting the linear region of the sample and reference plots to a model with common slope and individual intercepts.

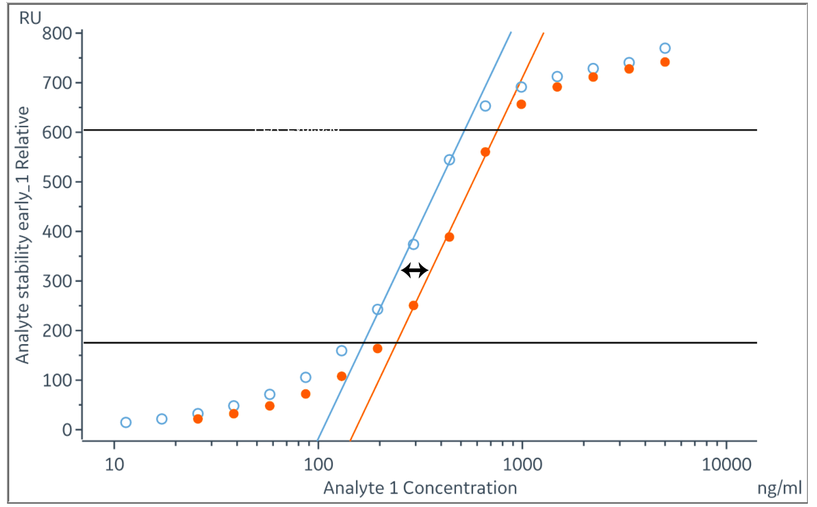
The common slope β is determined by fitting to the equation:

$$\beta = \frac{\sum_{1}^{n_R} (x_{iR} - \bar{x}_R)(y_{iR} - \bar{y}_R) + \sum_{1}^{n_T} (x_{iT} - \bar{x}_T)(y_{iT} - \bar{y}_T)}{\sum_{1}^{n_R} (x_{iR} - \bar{x}_R)^2 + \sum_{1}^{n_T} (x_{iT} - \bar{x}_T)^2}$$

Parameter	Description
β	Slope of the linear region
×, y	Log (conc) and response values respectively
R	Values for the reference substance
T	Values for the test substance

Relative potency is calculated from common slope and the average response and concentration values within the linear region:

$$\text{Relative potency} = \bar{x}_R - \bar{x}_T - \frac{\bar{y}_R - \bar{y}_T}{\beta}$$



For PLA evaluation, points that lie between adjustable horizontal boundaries are fitted to straight lines with common slope and individual intercepts. Relative potency is calculated from the horizontal distance between the lines.

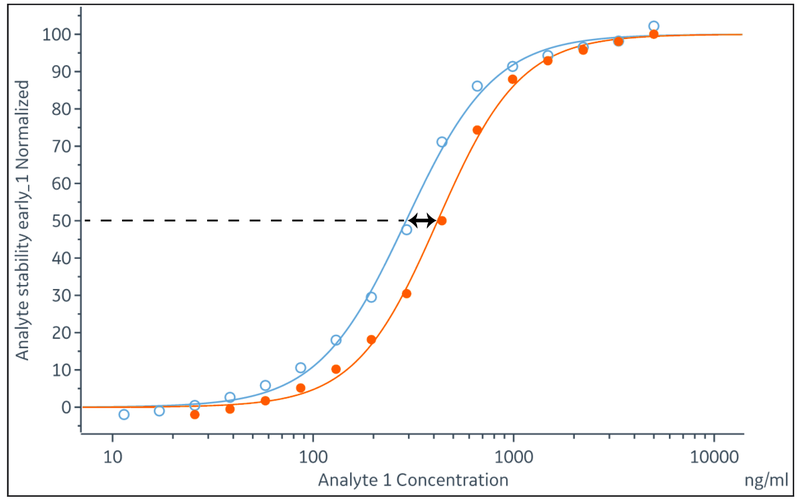
EC_{50} analysis background

EC_{50} analysis fits a four-parameter equation to the dose-response curve. The experimental data must provide sufficient points to cover both the upper and lower asymptotes.

Generic four-parameter fitting uses the following equation.

$$y = R_{hi} - \frac{R_{hi} - R_{io}}{1 + \left(\frac{x}{A_1}\right) A_2}$$

Parameter	Value	Description
x, y	Plot coordinates	—
R_{hi}	Upper asymptote	Fitted parameter corresponding to the maximum y-value
R_{lo}	Lower asymptote	Fitted parameter corresponding to the minimum y-value
A_1	EC_{50}	x-value at the inflection point of the fitted curve
A_2	Hill coefficient	Slope of the fitted curve at the inflection point



For EC_{50} evaluation, normalized dose-response curves are fitted to a 4-parameter function. Relative potency is calculated as the ratio of EC_{50} values for reference and test samples, expressed as a percentage.

Evaluation workflow

Potency determinations are currently supported only in Biacore Insight Evaluation Software with the **Concentration and Potency** extension. Result files containing concentration series measured in Biacore T200 can be imported into Insight and evaluated for relative potency. Potency evaluation of dose-response curves from other Biacore systems is not currently supported.

A suggested workflow for PLA and EC_{50} determination is summarized below. For more details, see the *Biacore Insight Evaluation Software User Manual.*

Step	Action
1	For runs performed in Biacore T200, import the result file into Insight Evaluation Software. Result files from Biacore 8K or Biacore 8K^{+} are accessible directly from Insight Evaluation Software.
2	Open the result file in Insight Evaluation Software using an appropriate evaluation method. Evaluation items for potency will be created provided that the content of the result file includes suitable positive controls that will be used as reference substances.
3	Define your reference substance(s) as positive controls in the **Variables** workspace.
4	Examine the results in the appropriate evaluation item (**PLA** or **EC_{50}**). Exclude any obvious outlying points from the evaluation. Adjust the boundaries that define the linear region in PLA evaluation if necessary. See the *Biacore Insight Evaluation Software Manual* for detailed descriptions of PLA and EC_{50} evaluation items.

Assessing the results: PLA

Evaluation by PLA relies on fitting parallel lines to the linear region of the dose-response curves for reference and test substances. By default, the linear region is set to 20% to 80% of the range between the estimated lower and upper asymptotes. This setting is usually suitable when the asymptotes of the dose-response curves are well-defined, but may need adjusting in other situations.

Drag the boundary lines in the Evaluation Software to adjust the range of the linear region. Use the 95% confidence interval in relation to the reported value for the relative potency (listed in the **PLA results** table) as an indicator of the reliability of the results.

Plot table | **PLA results** | PLA settings

Group	Control	Sample	Relative potency	95% confidence low	high	Common slope	Common R^2	Control slope	Sample slope	Control R^2	Sample R^2	Slope ratio S/C
Ch 1	sample curve	reference	138	124	153	911	0.991	912	910	0.992	0.990	1.00
Ch 2	sample curve	reference	134	111	165	973	0.990	943	1090	0.980	1.00	1.16
Ch 3	sample curve	reference	143	132	155	860	0.995	869	850	0.995	0.994	0.978
Ch 4	sample curve	reference	142	125	160	871	0.987	885	857	0.989	0.986	0.968
Ch 5	sample curve	reference	134	112	169	969	0.986	845	1090	0.979	0.993	1.29
Ch 6	sample curve	reference	136	119	153	915	0.988	939	890	0.990	0.985	0.947
Ch 7	sample curve	reference	144	126	163	1120	1.00	1160	1080	1.00	1.00	0.933

The **Slope ratio** parameter in the **PLA results** table indicates the degree of parallelism between the reference and test substances. A value of 1.00 indicates that the fitted lines are exactly parallel, and the substances are closely similar or identical in their binding behavior. Deviation from 1.00 indicates that the substances are not completely similar. The acceptance level for dissimilarity depends on the purpose of the experiment.

Note: Reducing the number of points in the linear region to the minimum of 2 for each curve will generally result in the closest fit to the experimental data, with correspondingly narrowest confidence interval. Take account of the number of experimental points in the linear region when assessing the reliability of the results.

Assessing the results: EC_{50}

Evaluation by EC_{50} determination relies on robust fitting of the 4-parameter function to the experimental data, which requires that the experimental points cover the full range of the dose-response curve:

- If the lower and upper asymptotes are not well defined, the fitting procedure may assign inappropriate values to the asymptotes. Use the **Restricted fit** function to minimize this issue (see the *Biacore Insight Evaluation Software Manual* for details).
- If the transition from the linear region to the asymptotes is not well defined, the reported values for the Hill coefficient (slope) and EC_{50} may be unreliable

Note that the evaluation will always report both relative potency and EC_{50} values, even if the experimental points do not cover the dose-response curve adequately.

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