

Calculating and evaluating ELISA data

Guidelines for calculating results from ELISA
data and statistical assay validation

Calculating results

Always run ELISA samples in duplicate or triplicate. This will provide enough data for statistical validation of the results. Many computer programs are now available to help process ELISA results in this way.

Calculate the average absorbance values for each set of duplicate standards and duplicate samples. Duplicates should be within 20% of the mean.

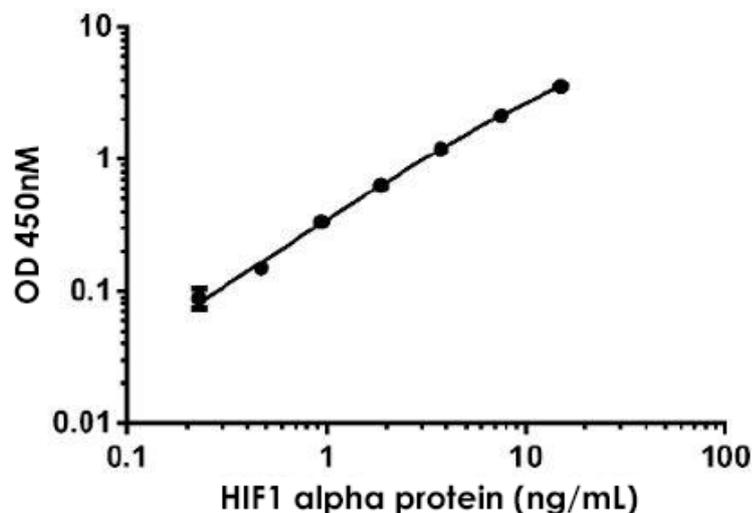
Standard curve

Create a standard curve for the target protein by plotting the mean absorbance (x axis) against the protein concentration (y axis). Draw a best fit curve through the points in the graph (we suggest that a suitable computer program be used for this).

We recommend including a standard on each ELISA plate to provide a standard curve for each plate used.

A representative standard curve is shown in the figure below, from human HIF1 alpha SimpleStep ELISA™ kit (ab171577). Each point on the graph represents the mean of the three parallel titrations.

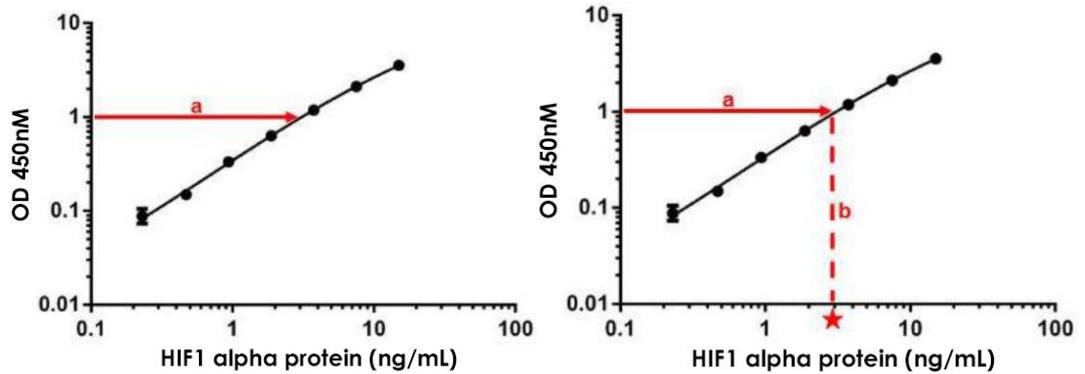
We recommend using a sample of known concentration as a positive control. The concentration of the positive control sample should be within the linear section of the standard curve in order to obtain valid and accurate results.



Concentration of target protein in the sample

To determine the concentration of target protein concentration in each sample, first find the mean absorbance value of the sample. From the Y axis of the standard curve graph, extend a horizontal line from this absorbance value to the standard curve.

For example, if the absorbance reading is 1, extend the line from this absorbance point on the Y axis (a):



At the point of intersection, extend a vertical line to the X axis and read the corresponding concentration (b).

Samples that have an absorbance value falling out of the range of the standard curve

To obtain an accurate result, these samples should be diluted before proceeding with the ELISA staining. For these samples, the concentration obtained from the standard curve when analyzing the results must be multiplied by the dilution factor.

Calculating the coefficient of variation

The coefficient variation (CV) is the ratio of the standard deviation σ to the mean μ :

$$C_v = \frac{\sigma}{\mu}$$

This is expressed as a percentage of variance to the mean and indicates any inconsistencies and inaccuracies in the results. Larger variance indicates greater inconsistency and error. Some computer programs can calculate the CV values from ELISA results.

High CV can be caused by

- Inaccurate pipetting; ensure pipette tips are sealed to the pipette before use so they draw up to correct volume of liquid
- Splashing of reagents between wells
- Bacterial or fungal contamination of either screen samples or reagents
- Cross contamination between reagents
- Temperature variations across the plate; ensure the plates are incubated in a stable temperature environment away from drafts
- Some of the wells drying out; ensure the plates are always covered at incubation steps

Spike recovery

Spike recovery determines the effect sample constituents have on detection of the antigen by the antibody. For example, the many proteins contained in tissue culture supernatant may hinder antibody binding and increase the signal to noise ratio, resulting in underestimation of the target concentration.

Known concentrations of protein are spiked into both the sample matrix and a standard diluent. The spiked protein is quantified using the assay and results from the sample matrix and the standard diluent are compared.

If the results are identical, then the sample matrix is considered to be valid for the assay procedure. If the recovery is different, then components in the sample matrix are interfering with the analyte detection.

What if a spike recovery experiment indicated that the sample matrix is affecting the results?

We recommend producing the standard curve using standard diluted in the sample matrix. Any effects on the results from the sample matrix will also be present in the standard, and therefore comparison between the standard curve and the samples is more accurate. Many of our ELISA kits contain a standard serum diluent for this purpose.

Another solution is to alter the sample matrix. For example, if neat biological sample is used, try diluting this in standard diluent. However, with this option, you will need to ensure that the dilution factor is taken into account when analyzing the results and that the concentration stays within the linear section of the standard curve.