ELISA

Standards
Controls
Results

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.

Positive and negative controls

- Running the appropriate controls helps you to accurately separate true positive results from potentially false results. Positive and negative controls will also be useful if you ever need to troubleshoot your protocol. Here we explain the various types of control samples you should use when running an ELISA.

Positive control

- Use either an endogenous soluble sample known to contain the protein you are detecting or a purified protein or peptide known to contain the immunogen sequence for the antibody you are using. A positive result from the positive control, even if the samples are negative, will indicate the procedure is optimized and working. It will verify that any negative results are valid.

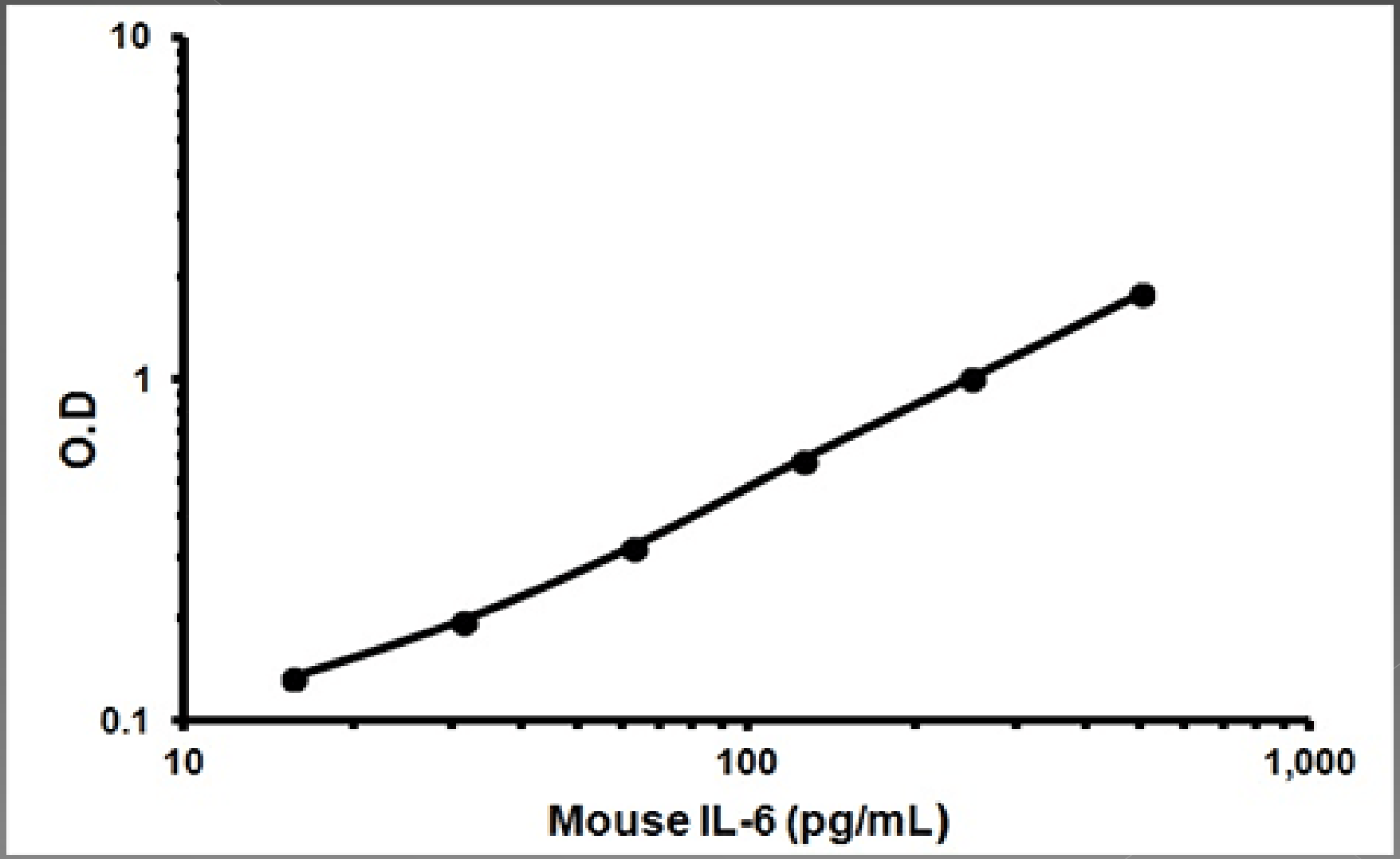
Negative control

- This is a sample that you know does not express the protein you are detecting. This is to check for non-specific binding and false positive results. Each plate you use should contain a negative control sample in order to validate the results.

Standard

- This is a sample that contains a known concentration of the target protein from which the standard curve can be obtained. A poor standard curve means the antibody didn't bind properly or doesn't capture the protein standard. The R^2 value of the trend line should be >0.99.

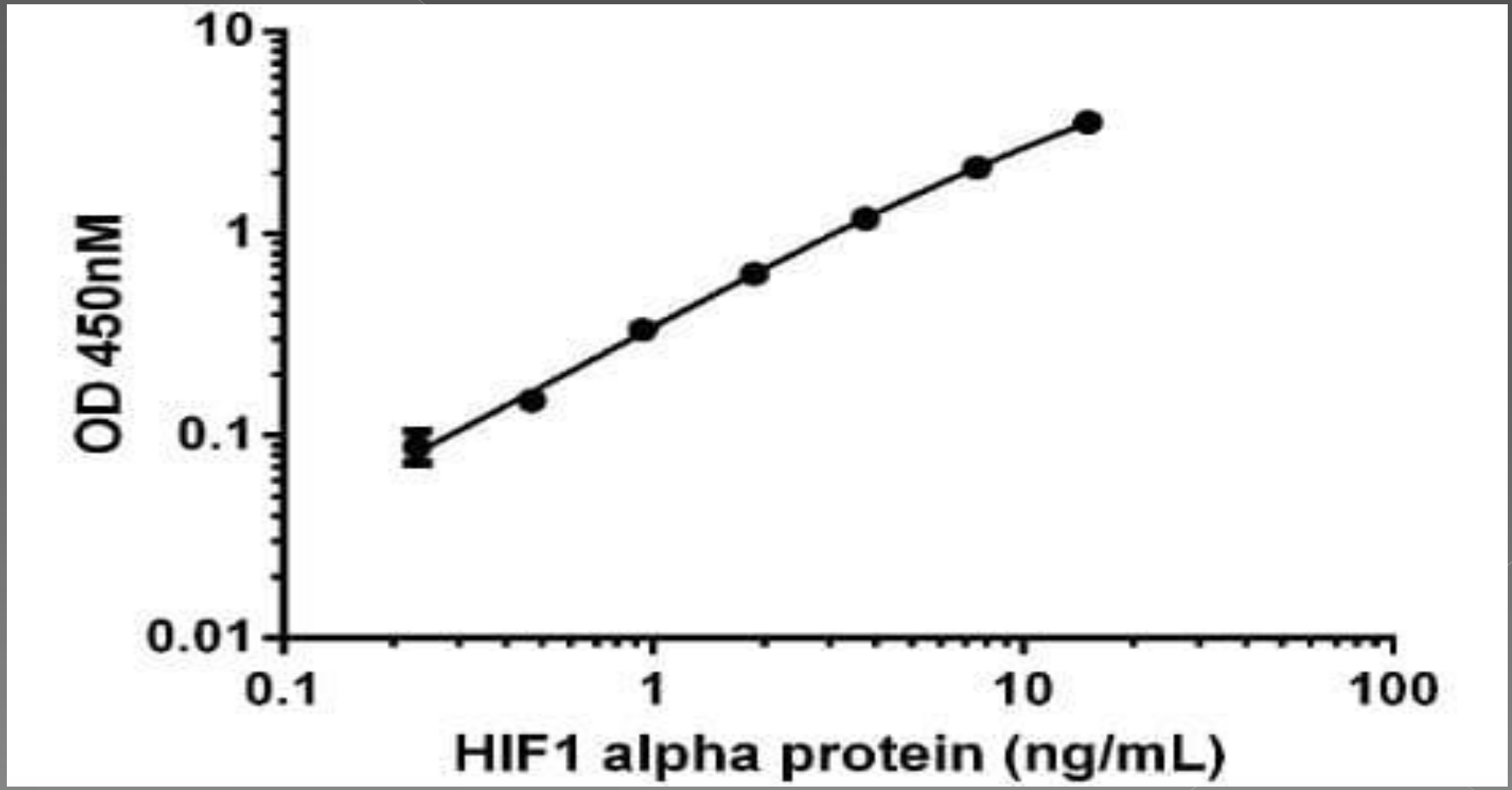
Standard



Standard curve

- Create a standard curve for the target protein by plotting the mean absorbance (y axis) against the protein concentration (x 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.
- 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.

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



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.

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 of 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.

Standard in sample matrix (spike control) control

- When testing serum samples in ELISA, include a standard in normal diluent buffer as usual. But we recommend to also include a standard diluted in serum from the species you are testing. The two can then be compared to ensure there is no effect on the standard curve from other proteins in the serum. This is known as a spike control and tells you that a target protein is recoverable after being spiked into a matrix. Acceptable results are 80–120%.

Endogenous positive control

- We recommend including an endogenous positive control if you are testing a recombinant protein sample. This should be an essential component of your experiment.
- There are inherent difficulties with antibody detection of recombinant proteins that need to be considered. Folding of the recombinant protein may be different from the endogenous native form, and may prevent antibody access to the epitope. This is particularly the case with tagged proteins. Always ensure tags are placed on the N or C-terminal end of the recombinant protein.
- Most importantly, always ensure the recombinant protein includes the immunogen sequence of the antibody you are using. An endogenous positive control is important to validate the results, as well as to indicate how well the reagents (eg antibodies) and procedure are working.

References

- https://www.abcam.com
- https://www.sinobiological.com
- https://www.bio-rad
- https://www.thermofisher.com