Calculating & Analyzing ELISA DATA

OVER 16,000 ELISA KITS FOR OVER 20 SPECIES

ASPIRE
TO CURE

ELISAGenie

CALCULATING & ANALYZING **ELISA DATA**

About ELISA Genie

ELISA Genie is a proprietary range of ELISA kits developed by Reagent Genie, a global life science reagents company based in London and Dublin.

Founded by **Colm Ryan PhD** and **Sean Mac Fhearraigh PhD,** our goal is to provide you with premium quality ELISA kits along with excellent technical and logistics support so you can maximise your success.

COLM RYAN PhD
CEO & co-founder of Reagent Genie

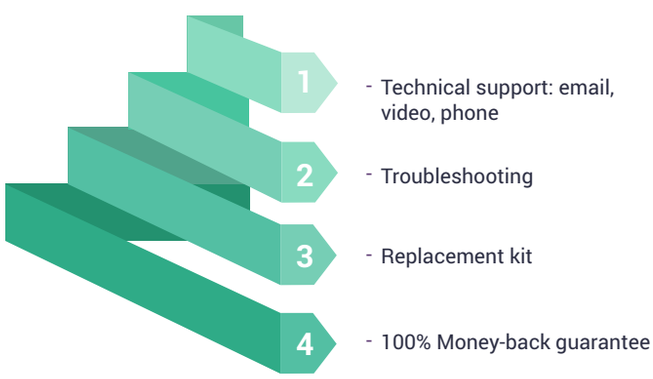
SEÁN MAC FHEARRAIGH PhD CTO & co-founder of Reagent Genie

Maximum Support & Guarantee

Antibody Genie provides excellence in support to all our customers!

Not only do we provide you with application based support before, during and after your experiments, but on those rare occasions when problems arise, we have a defined series of customer-centric steps to ensure that you are happy with our products.

So, don't worry, we also offer a 100% money-back guarantee should our products not perform as specified .



- Technical support: email, video, phone
- Troubleshooting
- Replacement kit
- 100% Money-back guarantee

Rapid Global Delivery

Whether you are served by one of our trusted local distributors or are part of our direct sales network, we endeavour to ship your products to you on-time, every time!

Contact us 24/7 on hello@antibodygenie.com to find our shipping times to your laboratory.

Key features & data provided with our **ELISA kits :**

Standard Curve | Spike & Recovery | %CV | Linearity | Range

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Recommended guidelines on calculating results from ELISA data and statistical assay validation.

When performing an ELISA assays it is good practice to run samples in duplicate or triplicate to ensure statistical validation of results.

Include positive and negative controls when setting up your ELISA plates.

Negative controls: Samples with no presence of your analyte
Positive controls: Samples with a known presence or quantity of your analyte

Depending on the type of ELISA used (qualitative, semi-quantitative or quantitative) data output will vary. Therefore you choose the specific ELISA you want to use based on the data that you want to analyse. Data is presented as a plot of optical density (OD) vs the log concentration of sample. Standards with known concentrations are used to generate a standard curve from which the concentration of an unknown analyte can be determined.

Preparing an ELISA Standard Curve

Once you have completed your ELISA protocol and analysed your samples & standards using a plate reader, you can plot your standard curve. In order to plot your standard curve for your analyte you plot the mean absorbance (Y axis) versus the known protein concentration of your standards (X axis). Use a computer to draw a best fit curve to plot your data.

Standards must be used on every ELISA plate to ensure consistency between data

Below to provide representative data from a sandwich ELISA and a competitive ELISA assay.

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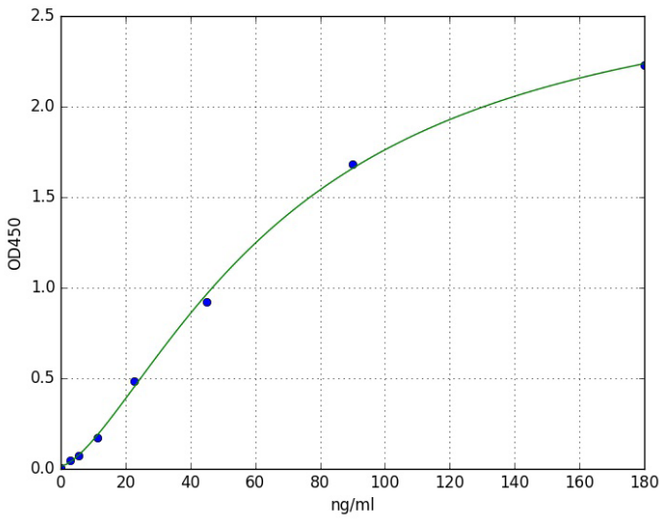
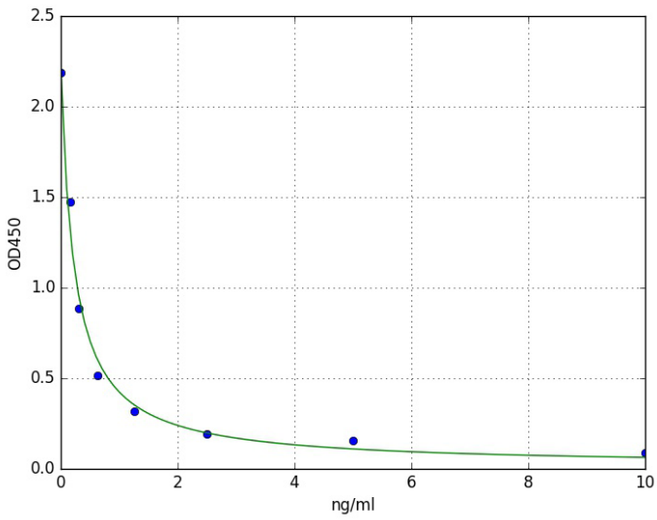


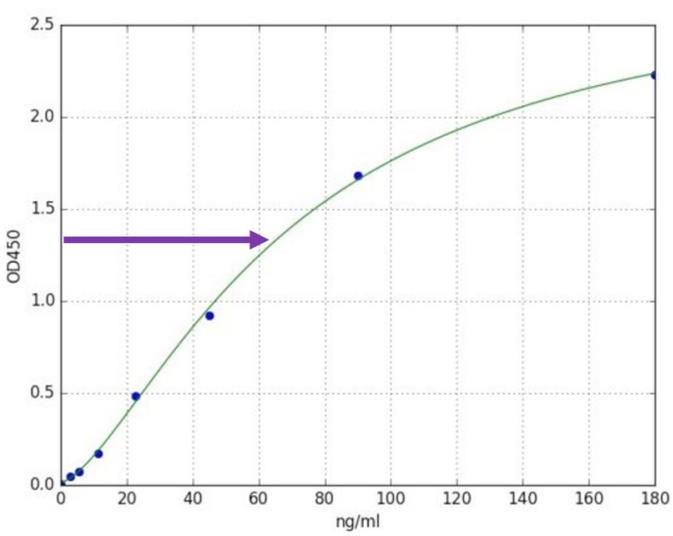
Sandwich ELISA Standard Curve: *Human CD8 alpha / CD8A ELISA Kit general standard curve for a sandwich ELISA assay. OD450 correlates with the amount of analyte (CD8A) present in the sample.*



Competitive ELISA Standard Curve: *Human Prepronociceptin / PNOC ELISA Kit general standard curve for a competitive ELISA assay. Reduction in OD450 value correlates with the amount of preponociceptin in the sample.*

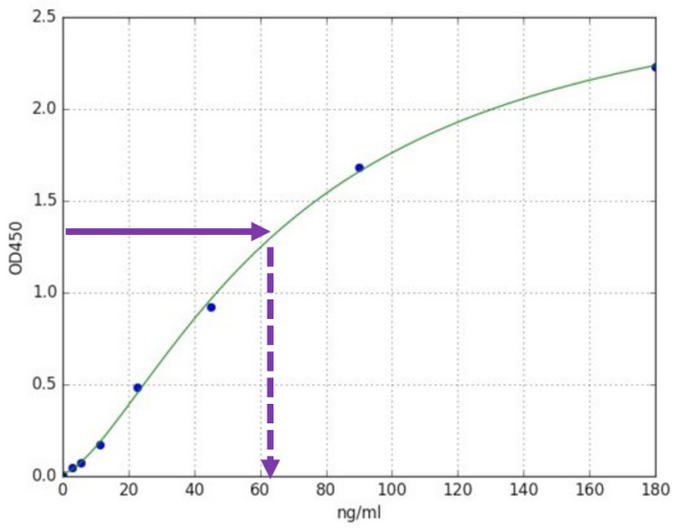
1. Calculating the absorbance

1. Calculate the average absorbance from duplicate/triplicate standards and samples. These should be within 20% of the mean.
2. Create a standard curve as described below.
3. Plot the mean absorbance (y axis) against the protein concentration (x axis) using excel or a similar suitable computer programme for standard samples.
4. Draw a best fit curve through the points on the graph.
5. From the standard curve graph extend a horizontal line from the absorbance plotted on the y axis to the standard curve.



To determine the concentration of target analyte 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.

6. Upon reaching the standard curve drop a vertical line down to read off the protein concentration.



At the point of intersection of your known absorbance with the standard curve, extend a vertical line to the X axis and read the corresponding concentration. Remember concentrations of analyte will need to be multiplied by the dilution factor of the sample.

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

2. Coefficient of variation

The coefficient of variation helps identify any inconsistences and inaccuracies in the results. It is expressed as a percentage of variance to the mean. The larger the variance the greater the inconsistency and error.

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

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

A High CV can be attributed to some or all of the following:

- Pipetting inaccuracies
- Sample contamination with bacteria/fungi or other reagents

- Temperature variation- plates should be incubated at a stable temperature away from drafts.
- Drying out of wells – plates should be covered during all incubation steps

At ELISA Genie each kit we produce is analysed to determine the sensitivity, range and inter/intra assay CV(%). This will ensure you know the full capabilities of our ELISA Kits including variability in advance of purchasing.

3. Spike recovery

Spike-recovery determines if any components in the sample interfere in antibody-antigen binding. The sample matrix is spiked with a known concentration of recombinant target protein. The ELISA is performed and the concentrations of protein determined from the standard curve. Recovery is typically presented as a percentage. Anything less than 80% generally means that components in the matrix are interfering in the ELISA and a different kit should be chosen

At ELISA genie we understand the need to show the accuracy of our ELISA kits but we also know how important it is to see if anything in the sample matrix interferes with antibody-antigen binding, a key step in ELISA. That is why we painstakingly do linearity and spike recovery analysis on every ELISA kit batch we produce.

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Horse ELISA kit assays

Goat ELISA kit assays

Cat ELISA kit assays

Rabbit ELISA kit assays

Dog ELISA kit assays

Sheep ELISA kit assays

Hamster ELISA kit assays

Mouse ELISA kit assays

Guinea Pig ELISA kit assays

Monkey ELISA kit assays

Bird ELISA kit assays

Porcine ELISA kit assays

Fish ELISA kit assays

Plant ELISA kit assays

Bovine ELISA kit assays

Rat ELISA kit assays

Chicken ELISA kit assays

Human ELISA kit assays

Vitamins, Hormones and Signalling molecules ELISA kit assays

Nous contacter

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