Technical Information

Simple ELISA Data Analysis

Suggestion for programming the cut-off in the microtiter plate reader

For each ELISA reagent in our catalogue, lyophilized positive and negative controls are available that are primarily intended to verify the assay performance. In most cases, OD readings are obtained that correspond to values which can be expected typically also for natural samples. However, pathogen concentration varies considerably and depends on a multitude of factors such as plant species and variety, season, physiological age, kind of tissue, storage, and extraction procedure. Therefore, we strongly recommend for any calculation of the cut-off to use your own positive and negative controls employing the same kind of tissue as you are analyzing.

Furthermore, depending on reagent, purity of chemicals, type of microtiter plate, handling (especially washing), and incubation conditions, background values may vary not only from reagent to reagent but also from plate to plate even within a series of plates testing the same pathogen. Determination of the cut-off should therefore be made individually for each plate.

There are many methods of calculating or setting the cut-off. In a previous information sheet «Technical Information - ELISA Data Analysis» we suggested to sort all data obtained from each plate individually in ascending order. In the resulting histogram, negative or background values could then easily be discriminated from potential positive values which were characterized by a sudden increase of the OD value. The values of the lower side of this «step» were then taken for the calculation of the cut-off using the formula «**mean value + 3 x standard deviation + 10%**». This method has the advantage that it discriminates potential positive samples, which have relative low OD values from evenly distributed negative or background values. The drawback is that this method is quite laborious to perform, relatively complicated and not applicable for programmable microtiter plate readers.

Alternatively, we can suggest another formula, which gives in most cases satisfactory results as well and is suited to be programmed in microtiter plate readers:

Add to wells A1 to D1 extracts of different healthy (negative) samples. The formula for the cut-off value can now be programmed as three times the mean value of these four wells:

Cut-off = mean value (of A1-D1) x 3

Interpretation of results: all values above this cut-off can be regarded as potential positive.

Restriction: The negative samples in wells A1-D1 should produce typically OD values in the range of 0.080 - 0.150 (after 60 min substrate incubation and blanking against air). If these values are > 0.150, we recommend to perform the data analysis using the more precise calculation «mean value+ 3 x standard deviation + 10%» described in «Technical Information - ELISA Data Analysis».

Caution: This «3 x mean value method» is less stringent than the more sophisticated method «mean value + 3 x standard deviation + 10%». In some cases, some samples with low OD values due to low pathogen concentration will be interpreted as negative with the «3 x mean value method» while being distinguished as low positive with the statistically more sophisticated method «mean value + 3 x standard deviation + 10%».

In any case and regardless which method you are ever choosing, we strongly suggest to repeat samples that are close to the cut-off.

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