Nordic BioSite - in Life Science Research

Determining Cross Reactivity with an ELISA

1. Determine the range of cross reactant to be run. This usually consists of a concentration 10 times greater than Standard #1 and ends roughly in the same area. (ie-PGE2 has a range of 5000 - 39.1 pg/mL, so a cross reactant range would start at 50,000 (10 x 5000) and end 50 pg/mL). Typically, run the dilution at 1:10 to cover the wide range needed. Also, make sure the first cross reactant is no higher than 25% ethanol (if the cross reactant is provided in an ethanolic liquid).

2. Dilute the cross reactant into the provided Assay Buffer in the kit. Once the range has been determined, the sample can be prepared by diluting into the Assay Buffer Assay Design's has provided in the assay. The cross reactant will be run as a sample. Typically, 100 ?'s are needed per well and each dilution of the cross reactant should be run in duplicate. Prepare the dilutions keeping this in mind.

3. Run the cross reactants in the assay. Once the standards for the assay and the cross reactants have been made, the assay can be run as directed in the associated kit insert.

4. Preparing the data. Once the assay has been run, the cross reactivity can be determined by subtracting the blank from the values. After the blanks have been subtracted, determine the average of all the duplicates for the standards and the dilutions of the cross reactant. When this has been accomplished, the next step is to divide these averages by the average Bo and multiply by 100. The resulting number should be a percent.

5. Graphing the results. With all of the standards and cross reactants divided by the Bo, the percents can be plotted on a % vs. Concentration graph. Draw a line across the 50% binding point and drop down vectors for where the assay's standard curve hit the 50% line as well as one for each cross reactant. Where the vector crosses the x-axis is the associated concentration. Determining % Cross reactive. Divide the standard curve 50% binding concentration by the 50% concentration of the cross reactant. Multiply this number by 100 and the result is "% Cross Reactive".

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