ARP blog – ELISA Troubleshooting tips – No signal

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This is the second in a series of blog posts looking at troubleshooting problems with ELISAs. This one is going to focus on the causes and possible to solution to no signal, or only a very weak signal, developing in your ELISA.

Since every step in an ELISA is pretty much key to it working, there are a lot potential causes for a lack of signal developing, and troubleshooting it is as much about checking how you followed the procedure as much as it is about the reagents.

Whereas in our previous post (poor standard curve) we extolled on the virtues of including quality control (QC) samples and their benefits in helping you troubleshoot, in the event of no signal developing at all, they're of little use.

No sample signal, but standards and/or QC samples fine.

If your standard/calibration curve and/or QC samples do have a signal, but your samples don't (when you would expect them to), then you can be sure there is something erroneous with your samples. There are a couple of possible problems.

- ***Using a new sample matrix***

Not all ELISA's work with all samples types. For example, an ELISA developed to use cell culture media or lavage probably won't give reliable results if you switch to serum or plasma.

If you've changed sample types you may need to consult the manufacturer, or re-develop your ELISA to work with the new sample type.

- ***Dilutions too high***

A simpler explanation could be that your sample have been over diluted, beyond the sensitivity of the ELISA.

If you keep all of your sample dilution tubes until the end of the assay, you may be able to tell if and where an error may have crept in by looking at the remaining volumes. Alternatively, double check your dilution calculations.

- **Analyte below detection limit**

Another possibility may be that your samples simply do not contain enough of the analyte; that their below your detection limit.

No signal at all

Below are listed a number of possible causes for zero signal development.

Depending on how you document your assays, some may be easy to spot as the culprit, whereas others it will be hard to discern between. Because of this, as with other techniques, when repeating an ELISA be sure to start with freshly prepared reagents if possible, double checking expiry dates and storage conditions, as this will help negate errors in previously prepared reagents from causing the same problems.

- ***Incubations times/temperature***

Double check your incubations times and temperatures and ensure they are in line with the manufacturers guidelines. Too short or too cool an incubation could affect the assay kinetics leading to no signal developing. Long and cool, or short and warm tend to be the most common.

- ***Insufficient/incorrect capture/detection antibody***

This is likely to be most common with in-house developed ELISAs, where you're preparing these reagents yourself for each assay. Double check dilutions and, again, if you keep your dilution tubes, check the volumes to see if there could be an error there.

Where a manufacturer provides 'ready to use' reagents, double check that they have been used in the correct order.

- ***Not enough capture/detection reagent added***

Double check the correct volumes of reagents were added to the plate, or ensure you're following the manufacturers guidelines. Alternatively, you could increase the concentration or volume of the detection reagent.

- ***Reader wavelength***

Double check your software/plate reader to make sure the correct wavelength was used for the substrate you're using. If using a detection reagent that needs to be 'stopped' visually inspect the plate to ensure this has been done.

Double check the plate reader hasn't been left set up for one substrate when another was used.

E.g TMB is blue with absorbance maxima at 370 nm and 652 nm, but when the reaction is stopped with acid, the maxima is 450 nm, while if ABTS is being used the maxima are 410 or 650 nm.

- ***Substrate solution***

Ensure that the correct substrate has been used and prepared immediately before use. Double check expiry dates of any stock solutions.

- ***Plate washing***

If you're plate washing is too vigorous or too long, you may have washed all of your sample analyte/reagent off the plate. Double check automated plate washer settings.

- **Dry wells**

Letting your plate dry out can cause all sorts of problems in an ELISA that are difficult to predict. You might get 'flyers' (wells with anomalously high values) or no signal at all. Be careful to ensure that your plate is never left to sit long enough to dry out.

As you can see, there are plenty of possible places for an error to creep into in assay, especially if it's one you're unfamiliar with, but we hope that this troubleshooting guide will get your assay back up and running again as soon as possible.