ELISA Troubleshooting Guide

Be sure to read the kit brochure carefully before running any assay. The timing and volumes can be different from assay to assay. Following the kit assay protocol and sample extraction methods is critical practice. Any deviation from the protocols in the brochures may lead to undesirable results. Overall, the assay operator should strive for consistent, reproducible results in a clean laboratory environment.

A.) Pipetting errors are one of the major sources of error in immunoassay methodology. To help improve dispense precision and timing as well as kit accuracy, we recommend the following.

- Run calibrators and samples in replicates.
- Use a positive displacement pipette to dispense calibrators and samples with a diluent that contains more than 10% methanol.
- Use a multichannel pipette to add conjugate, antibody, substrate and stop solution in a plate assay when multiple strips are used in a single run.
- Use a repeater pipette to add conjugate, antibody, substrate and stop solution to tube assays when more than 5 tubes are used in a single run.
- Use clean pipette tips for all reagents to avoid cross contamination.

B.) Temperature errors are another major source of error in immunoassay methodology. To help improve assay accuracy, we recommend the following.

- Run all assays in the same location/temperature.
- Allow all reagents to reach room temperature 20°C to 28°C (62°C to 82°C) prior to use.
- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).

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C.) Timing and mixing are also important when running assays. The operator should do the following.

- Use a timer to keep track of incubation times.
- Be sure to shake plates/tubes to thoroughly mix reagents for at least 30 seconds.
- Take caution not to spill reagents within the plates/tubes during shaking.

D.) Improper washing of the plate wells/tubes may impact assay results. When washing wells/tubes be sure to.

- Use the proper wash solution as detailed in the brochure.
- Flood the wells/tubes completely and immediately decant the wash solution. Do not soak.

E.) When it comes to sample extraction, it is important to follow the kit-specific sample extraction protocol provided by Beacon. A major cause of error with samples stems from extracting samples using different techniques than recommended by Beacon. If you experience problems with samples, it is important to consider the following.

- The final sample extracts are in the same diluent as the calibrators (this should not be a problem if following the Beacon protocol).
- Cross reactivity as stated in the kit's brochure.
- Samples found to have or expected to have concentrations greater than the kit's highest calibrator should be diluted prior to analysis.
- When diluting calibrators, be sure to double check calculations.
- Samples outside of the calibrators B/B_0 ranges must be reported as less than the lowest calibrator value or greater than the highest calibrator value.

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F.) After running your assay, it is important to calculate your results as described in the brochure. If the Optical Density (OD) of the zero calibrator is low, please consider the following:

- Were the kit components warmed to room temperature prior to use?
- Were the wells/tubes completely flooded when washing?
- Were the wells/tubes contents decanted in between each wash?
- Were the wells/tubes soaked prior to decanting?
- Was the correct wash solution used as detailed in the product brochure?
- Were the inverted wells/tubes tapped on an absorbent material after the final wash step to remove excess wash solution?
- Were reagents from different lots used/mixed?
- Were the reagents expired?
- Were the contents of the wells/tubes mixed by gently shaking before incubation?
- Were the wells/tubes read immediately after the addition of stop solution?

G.) If poor CVs were seen when analyzing the data, consider the following as potential causes:

- Ensure the OD of the zero calibrator was not < 0.6. A low OD will usually generate a larger CV.
- The pipettes used to run the assay were calibrated routinely and the appropriate size/type, clean pipette tips were used.
- Ensure you are familiar with the equipment used to run the assay. It is recommended to practice pipetting before delivering small volumes, especially when gloves are worn.
- The reagents were dispensed using a pipette capable of delivering the required volume with accuracy.
- A positive displacement pipette was used to dispense calibrators and samples if the diluent contained methanol.
- A multichannel pipette was used to add conjugate, antibody, substrate and stop solution if multiple strips were used in a single run.

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- If a multichannel pipette was used, ensure that all tips were in place and the level of the reagents in the tips were even.
- A repeater pipette was used to add conjugate, antibody, substrate and stop solution if more than 5 tubes were used in a single run.
- Reagents were added in a consistent fashion.
- Ensure there were no scratches or debris on the optical surface. This can be remediated by gently wiping the optical surface with a lint-free wipe.
- The work area was clean. It is possible that unwanted, foreign substances in the well/tube may react with the substrate or block the light path.

If you require further technical assistance, please contact Beacon using the information below.

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