Low Absorbance Values

Low ODs or absorbance values can be due to a number of problems as discussed below. To determine the expected absorbances for your particular kit lot please refer to the Certificate of Analysis included with each shipment. The COA will show the absorbances obtained by our QC laboratory for the zero standard and the highest kit standard. Some variation in absorbance values is expected from lab-to-lab and technician-to technician. Provided you are obtaining acceptable precision and sensitivity, ODs lower than what is given on our C of A are acceptable.

1. Washing technique or equipment – Use of automated plate washers or hand-held vacuum aspiration devices could significantly dissociate specific bound analyte. Washing more than 4 times or soaking the wells in wash solution for any period of time can likewise lower expected ODs. Use of wash solutions other than the one provided with the kit may reduce your ODs. Review the kit package insert for proper washing technique. Visit our web site www.cygnustechnologies.com to view a video of how we perform plate washing. Do not be overly aggressive in banging the plates to remove all residual liquid. It is not necessary to remove all liquid. The 4 washes will sufficiently dilute unbound material such that less than 3µL of residual liquid at each wash step will not significantly add to background ODs after the 4th wash.

2. Shaking of the plate during immunological incubations – Placing the plate on a microtiter plate shaker can significantly accelerate the rate of binding. As various shaker instruments can have somewhat variable speeds and range of motion, the magnitude of shaking effect can vary. We have determined that the optimal rate of shaking is in the range of 400 to 600 rpms for most assays and most plate shakers. If ODs are lower than expected you may consider increasing the speed of shaking. Be advised that speeds greater than 600 rpm risk spilling liquid from well-to-well or causing it to make contact with the material used to cover/seal the plate resulting in very poor precision. If you do not shake the plate but instead perform the immunological incubations without agitation it may be necessary to increase the incubation time by up to 2-fold to achieve the same absorbances that are seen with shaking.

3. Laboratory or reagent temperature can have a significant effect on assay ODs. The QC of our kits takes place at 25°C. If your laboratory is colder than this, it may be necessary to extend incubation times in order to achieve higher ODs. Alternatively, you may use an incubator set at 25°C. Make certain that the kit reagents are up to room temperature prior to pipetting.

4. For HRP assays with TMB substrate, the plate should be read within 30 minutes after addition of stop solution since color will fade over time.

5. Check plate reader for proper performance. The instrument filters for both the test and reference wavelengths can deteriorate over time.

6. Carefully review the kit directions insert for proper technique. **When possible have another technician in another laboratory and with different equipment perform the assay. This is often the best way to identify the cause of low ODs.**

7. If you would like higher ODs than those shown in the C of A it is often possible to simply extend the incubation time of one or all of the various assay steps. Contact our Technical Service Department (techsupport@cygnustechnologies.com) for advice on how best to modify the assay protocol.