



Low Absorbance Values

Low OD 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. This COA will show the absorbances obtained by our QC laboratory for the zero standard and the highest kit standard.

1. Washing technique or equipment – Use of automated plate washers or hand held vacuum aspiration devices may 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. Using wash solutions other than the one provided with the kit may reduce your ODs. Review the kit product insert for the proper plate washing technique. Specific information can be found at [Washing of Microtiter Wells](#) or watch the [Washing Technique for Microtiter Plate ELISA Video](#) to view the washing technique we recommend.
2. Laboratory or reagent temperature can have a significant affect 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 at to room temperature prior to pipetting.
3. For HRP assays using TMB substrate, the plate should be read within 30 minutes after addition of Stop Solution since color will fade over time.
4. Check your pipets and plate reader for proper performance.
5. Carefully review the kit product Insert for proper technique. When possible have another technician in another laboratory and with different equipment perform the assay to identify the cause of low ODs.
6. Our protocols call for the use of an approved microtiter plate shaker. Shaking will increase binding by overcoming analyte diffusion rate limitations involved in capture onto the antibody coated microtiter plate. If you do not have a microtiter plate shaker it will be necessary to extend immunological incubation times by as much as two fold to achieve the same ODs as with shaking.
7. If you seek higher ODs than those shown in the COA, it is often possible to simply extend the incubation time of one or all of the various assay steps. Contact our Technical Service Department for advice on how best to modify the assay protocol.