AntiCoV-ID™ IgG ELISA
Technical FAQs

Frequently Asked Questions (FAQs) - Technical
• **The Instructions for Use (IFU) doesn't mention the use of shaking in the assay test procedure. Should I be shaking during my sample incubation and/or enzyme conjugate incubation steps?** No. Akston developed and validated the kits to be used without shaking, and shaking in any of the steps will result in increased ODs, larger CVs, and failed assay runs. In some older versions of the IFU the optional use of a plate shaker was listed in error. Newer production lots of kits have corrected this error in the IFU.
• **I am manually washing the plates in the assay kit, and the assay kit background signal is higher than desired. What can I do to improve (lower) the background signal?** Akston's AntiCoV-ID™ IgG ELISA was developed to have very low signal background. While manual washing of plates should work fine, Akston recommends the use of an automated plate washer with plate agitation during each wash cycle where possible. If washing manually, we suggest tapping the plate gently (e.g. against the palm of your hand) a few times during each wash cycle. Ensure that wash buffer is completely decanted between washes, and tap the plates dry (e.g. on paper towels) after the final wash cycle to remove any liquid remaining in the wells.
• **We currently run assays in duplicate in my laboratory, but we are considering looking at serological assays that can be run in single well analysis. Does the Akston AntiCoV-ID™ IgG ELISA allow for this?** Yes. The Akston AntiCoV-ID™ IgG ELISA was validated using both duplicate well and single well analysis modes.
• **The kit Instructions for Use (IFU) details using 4-PL regression for fitting the calibrator (standards) strip data. Should I use a weighted or non-weighted 4-PL model to fit the data?** Akston recommends using a 4-PL regression with no weighting.
• **I am using an automated ELISA platform for running the AntiCoV-ID™ IgG ELISA. Will the kit work on my automated ELISA equipment?** Yes, several of our customers have successfully run and validated the assay on automated platforms. In some cases, extra kit reagents were required. For more information about obtaining extra reagents, please contact your local Mercodia sales representative.
• **I see that heat-inactivated serum or plasma may be used with this assay. If I heat-inactivate my samples, can I still use the positive/negative cutoff value listed on the back of my IFU?** No. The positive/negative assay cutoff value was obtained through validation and additional quality control studies with the assay kit using only non-heat inactivated samples. However, as described in the IFU, supportive, but non-validated studies have been conducted on heat-inactivated serum and plasma. The results have shown that good assay performance can be obtained, but serum background signal is increased. Therefore, the assay cutoff value must be revised for heat inactivated samples. Akston strongly encourages those wishing to use heat-inactivated samples to re-validate the assay cutoff value with samples prepared according to the customer's laboratory's heat inactivation protocols. Please contact the Akston technical team who will assist in your validation efforts with heat-inactivated serum or plasma.

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- **The Assay Control 1 and/or Assay Control 2 are failing high on my assay. What may be causing this?**
  The Assay Controls 1 and 2 are included on the plate to identify instances where the assay run strayed from the parameters under which the kit was validated, particularly with respect to the standard curve development. Often, the main reason for this phenomenon appears to be stopping the TMB substrate development time too early. The kit IFU indicates the use of 25 min of development time, which was the TMB development time used to validate the assay at Akston's facility. Experience with external customers has shown that, in some cases, variations in manual vs. automated washing, wash protocols, laboratory temperatures, etc. can influence the development of the assay and assay control values. In cases where the assay controls are failing a bit on the high side, Akston recommends developing the assay in the TMB step for up to 30-32 minutes as needed so that the OD450 values obtained for the standards after stopping of the assay compare well with those shown in the Example Values section below.

Example Values for Standards (see top right of page for applicable lots)

The absorbance values in the table below were collected during 3 independent runs, conducted over multiple days. These values are for reference only, and should not be used for the analysis of data.

Std (Wells)	OD (450 nm)		
	Run1	Run2	Run3
Std1 (A1,A2)	3.140	2.997	3.127
Std2 (B1, B2)	2.411	2.316	2.407
Std3 (C1, C2)	1.512	1.473	1.531
Std4 (D1, D2)	0.850	0.819	0.887
Std5 (E1, E2)	0.409	0.409	0.443
Std6 (F1, F2)	0.204	0.201	0.226
Std7 (G1, G2)	0.124	0.120	0.124
Std8 (H1, H2)	0.044	0.043	0.044