



Poor Recovery of Protein A

Some labs have reported poor (under-recovery) of spiked Protein A into their samples even when following the sample treatment steps as described in the [product insert for Catalog #F050H](#) and the [product insert for Catalog #F400](#). We have determined the most common cause of this poor recovery is due to incomplete processing of the sample to remove the interfering product antibody. Small quantities of some product antibody remaining in the supernatant after the sample treatment can re-bind Protein A or otherwise interfere in the detection of residual Protein A by the ELISA. Below are some recommendations to eliminate this interference:

1. Make certain your centrifuge is very well balanced. An out-of-balance centrifuge will cause the pellet to be disturbed thus re-suspending the interfering product antibody. If you can feel or hear vibration particularly as the centrifuge accelerates or decelerates your centrifuge is likely out-of balance. This could be caused by rotor deterioration or variable volumes/weights in the tubes being centrifuged. Even a 25 μ l volume difference from one tube to the next will create significant vibrations in the centrifuge step. Make certain your microfuge tubes are of the same type and weight.
2. Higher rates of centrifugation may yield a more tightly packed pellet that is less prone to re-suspension. The sample processing protocol calls for centrifugation of at least 6000 rpm. However, you may improve recovery by centrifuging faster if allowed by your centrifuge. We have centrifuged at speeds up to 15,000 rpm without experiencing problems.
3. The interfering product antibody pellet can be accidentally re-suspended by mishandling after removal from the centrifuge. Avoid bumping the tube. Also pipet the supernatant sample from the tube into the microtiter wells immediately after removal from the centrifuge, to prevent the pellet from re-solubilizing.
4. Some customers have reported that extending the heating step from 5 minutes to 10 minutes improves recovery for more problematic antibodies. Provided you follow the same procedure for the kit standards as well as your samples, we have seen no problem with increasing the heating time.
5. Simple dilution of the sample will overcome most sample interference factors. As long as the dilution does not reduce the levels of Protein A below the detection limits of the assay. We recommend dilution of your sample in our Sample Diluent Catalog #1028. For most product antibodies significant interference is eliminated after dilution of the product antibody to a concentrations in the range of 0.1 to 1mg/mL. **It is important that all sample dilutions be performed prior to the sample treatment step!**

If you still have problems with recovery after utilization of the methods above, please view the video on [Protein A Sample Treatment Procedure](#), or contact our Technical Services Department for advice on how to proceed.