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Protein A-h ELISA kit
Catalog # F050H
Sample Denaturation Procedure Modification (Boiling Step)
Validation Report
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Summary: The original F050H kit used only an acid treatment step to dissociate the Protein A bound to the product antibody so that the kit anti-Protein A antibodies could detect it accurately. This procedure was shown to be accurate for all antibodies at concentrations less than or equal to 1mg/mL. At higher product antibody concentrations, some customers reported under-recovery of Protein A spiked into their antibody. These customers did not want to dilute their antibody further believing it would significantly compromise the sensitivity of detection for Protein A. In response to this concern, we investigated other ways to overcome product antibody interference that would not require as much dilution of the sample. The addition of a boiling step after the acid dissociation, was shown to remove the product antibody without affecting the subsequent recovery of Protein A. Such a procedure better insures the accuracy and robustness of the assay. The following study was performed to validate this protocol change. The only change required to the previous F050H kit is a new kit directions insert to reflect the addition of the boiling step. All other reagents and manufacturing processes remained the same and thus the validation study is abbreviated from what would be required if kit components were changed. Customers may continue with the old non-boiling protocol if they do not wish to validate the new protocol.

Materials:
- Protein A Standards
- Biotinylated anti-Protein A (Product # F051H) lot 5010B
- Streptavidin:Alkaline Phophatase, Lot 5010
- Anti-Protein A coated Strips, Lot 13030
- Various product antibodies and hIgG preparations as indicated

Methods:

Sample Treatment Procedure:

1. Dilute all samples including the standards and control by adding 1 part of sample denaturing buffer (Cat #F054) to 4 parts of sample. Mix by vortexing. (For example: pipet 50µL of F054 into a microfuge tube containing 200µL of sample.)
2. Make a small pin or needle hole in the cap of each microfuge tube to allow for venting of heated air inside the tube.
3. Place tubes in a 100°C heat block or boiling water bath for 5 minutes.
4. Remove tubes, allow to cool for 5 minutes, and then centrifuge at ~6000 x g for 5 minutes in a microfuge or other adapted centrifuge.
5. Proceed to assay protocol.

Assay Procedure: The actual assay is unchanged from the one used before the addition of boiling to the sample preparation step.

1. Pipette 100µL of biotinylated anti-Protein A (F051H) into all wells.
2. Pipette 50µL of supernatant from the denatured samples, standards, and controls into wells indicated on the work list.
3. Transfer to microtiter plate shaker at 180rpm and incubate at room temperature for 1 hour.

4. Aspirate, wash with at least 300µL of diluted wash solution and aspirate. Wash and aspirate a total of 4 times.
5. Pipette 100µL of Streptavidin:Alkaline Phosphatase (F009) into each well.
6. Transfer to microtiter plate shaker at 180 rpm and incubate 1 hour at room temperature.
7. Aspirate and wash a total of 4 times as in step 4 above.
8. Pipette 100µL of substrate (F008) into each well.
9. Incubate for 30 minutes.
10. Read absorbance at 405/495nm and calculate results.

Data References: Raw data for these experiments are recorded in Notebook #2-Protein A/B12 pages 75-89.

ELISA Validation: Typical absorbance data for the kit standards tested in duplicate and using the new protocol are shown below in Table 1.

Table 1
Typical Protein A ELISA Standard Curve

Standard Concentration	Absorbance 1st well	Absorbance 2nd well	Mean absorbance	% CV
0 (ng/mL)	0.070	0.073	0.072	2.9
0.25	0.123	0.120	0.122	1.7
1	0.210	0.219	0.215	3.0
4	0.596	0.601	0.599	0.6
16	1.691	1.761	1.726	2.9

Recovery/Matrix Interference: Defined as the ability of the assay method to correctly quantitate known concentrations of Protein A in a given sample matrix, accuracy was evaluated by spiking Protein A into various human antibody preparations. Recommended acceptable limits of recovery are +\- 20% of the added Protein A. Table 2 shows the recovery in spikes ranging from 2 to 16ng/mL of Protein A into 3 different hIgG samples: a normal hIgG preparation isolated from human serum, and 2 previously problematic customer antibodies at 4 and 1.6mg/mL, that did not yield acceptable recovery with the old non-boiling protocol. This data demonstrates that the boiling protocol gives acceptable recovery in all 3 preparations. This does not ensure that all antibody preparations will be yield accurate recovery of Protein A levels, and thus each user is cautioned here and in the kit directions insert to evaluate their sample matrices for recovery in a similar experiment before reporting results with this assay.

Table 2
Recovery in Various Sample Matrices

Sample Matrix	Protein A Spike	% Recovery
#1 2mg/mL normal hIgG	16ng/mL	98
#2 4mg/mL normal hIgG	16ng/mL	83
#3 Customer Ab @ 4mg/mL	8ng/mL	105
#4 Customer Ab @ 4mg/mL	4ng/mL	90
#5 Customer Ab @1.6mg/mL	4ng/mL	110
#6 Customer Ab @1.6mg/mL	2ng/mL	95
		Average recovery = 96.8

Precision: Because there was no change to the assay protocol a complete precision study profile was not performed. Precision is defined in this study as the average % coefficient of variation on duplicate sample OD values. The average %CV on duplicate samples over 6 assays was 2.3%. This is excellent precision and consistent with the results obtained with the non-boil sample preparation. Because actual precision may vary from laboratory to laboratory, and technician to technician it is recommended that all operators demonstrate acceptable precision before reporting results.

Sensitivity: The Protein A concentration corresponding to a signal 2 standard deviations above the zero standard is defined as the limit of detection (LOD). This was determined by averaging the standard curve results over 6 assays. The LOD thus determined was ~100pg/mL.