



Protein A ELISA Kit

1 Plate Kit Catalog # AB000109

Complete kit for the determination of residual Protein A contamination in bulk products purified by Protein A affinity processes.

Please read this insert completely prior to performing the assay.

This kit is intended for research use only. Not for use in diagnostic procedures.

Assay Principle

The Protein A ELISA kit is designed to quantitatively measure residual Protein A contamination in bulk products after Protein A purification processes. Please read the complete kit insert before performing this assay. The assay utilizes an assay plate which has been pre-coated with rabbit anti-Protein A antibodies. The test samples along with the control samples are pre-treated by heat denaturation to remove IgG and IgG-like proteins. It is important to remove IgG proteins prior to performing this assay as Protein A – IgG complexes (which are very stable) can prevent recognition of Protein A by the capture antibody on the plate. After the immobilized antibody captures residual Protein A present in the samples, the captured Protein A or Protein A like proteins is detected by biotin labeled detection antibody, followed by addition of Streptavidin peroxidase conjugate. This conjugate binds strongly to the biotin molecule on the detection antibody. The level of bound peroxidase conjugate present in each assay well is determined by addition of a Peroxidase substrate (TMB), which is acted upon by the peroxidase enzyme to generate a blue coloration. After a short incubation (10-15 minutes), the reaction is stopped by the addition of acid solution that generates a yellow coloration. The absorbance of each well in the assay plate at 450 nm is then recorded using a suitable plate reader.

A series of Protein A standards are provided to generate a standard curve for the assay and all unknown sample concentrations should be read off this standard curve. It is not necessary to boil these standards as they are already free of contamination IgG molecules.

Supplied Components

Coated Clear 96 Well Plates

A clear plastic microtiter plate(s) coated with Rabbit anti-Protein A IgG.
Kit AB-000109 (1 plate)

Protein A Standards

Protein A standards diluted to seven concentrations in a special stabilizing solution, sufficient for generating a standard curve from 8 ng/ml to 0.125 ng/ml (made from a two-fold serial dilution).

Kit AB-000109 (eight tubes, varying concentrations including blank each with 350 μ L / tube , the 8 ng/ml standard has 850 μ L/tube, the additional sample is used for spike recovery).

5x Dilution Buffer

Buffer used for dilution of reporting antibodies and Streptavidin-HRP conjugate. The 15 ml of concentrate should be diluted to 75 ml with 60 ml of deionized or distilled water.

Kit AB-000109 (15 ml). **Please make sure that the testing samples are diluted with PBS-T to 0.5 mg/ml not this dilution buffer.**

10x PBS-T

After dilution, this is used for wash solution (PBS with 0.1% Tween-20) and dilution of testing samples. The 30 ml of concentrate should be diluted to 300 ml with 270 ml of deionized or distilled water.

Kit AB-000109 (30 ml)

Reporting antibody

A biotin labeled Rabbit polyclonal antibody specific for Protein A. Immediately prior to the assay, dilute the entire 150 μ L into 15 ml of 1x Dilution buffer to give a 5 μ g/ml working stock.

Kit AB-000109 (0.5 mg / ml, 150 μ L / tube)

Streptavidin-HRP Conjugate

A Streptavidin – Horse Radish Peroxidase conjugate in a special stabilizing solution. Immediately prior to the assay, dilute the entire 375 μ L into 15 ml of 1x Dilution buffer to give a 0.1 μ g/ml working stock.

Kit AB-000109 (4 μ g / ml, 375 μ L / tube)

TMB Substrate

Use directly without dilution.

Kit AB-000109 (15 ml)

Stop Solution

A 1M solution of sulfuric acid. CAUSTIC. Use directly without dilution.

Kit AB-000109 (15 ml)

Plate Sealer

Kit AB-000109 (one)

Other Materials Required

Distilled or deionized water.

Single- and multi-channel micro-pipettes with disposable tips to accurately dispense volumes 5-250 μ L.

Plastic tubes (i.e. 1.5 ml) for sample dilution

Reagent reservoirs for sample addition

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4-parameter) fitting. Contact your plate reader manufacturer for details.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 2

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

Procedural Notes

Allow diluted reagents and buffers to reach room temperature (18-25°C) prior to starting the assay. Once the assay has been started, all steps should be completed in sequence and without interruption. You do not want the plate to dry out in between steps as this can cause high backgrounds or erroneous results. Make sure that required reagents and buffers are ready when needed. Prior to adding to the plate, reagents should be mixed gently (not vortexed) by swirling.

Avoid contamination of reagents, pipette tips and wells. Use new disposable tips and reservoirs, do not return unused reagent to the stock bottles / vials and do not mix caps of stock solutions.

Incubation time can affect results. All wells should be handled in the same order for each step.

Microplate washing is important and can affect results by giving erroneous results or high backgrounds. We recommend a multichannel pipette to add 250 µl of buffer to each well across the plate, followed by a dumping out of contents (to a sink or other receptacle) with a rapid wrist motion. The plate should then be tapped firmly on a paper towel to shake out any remaining liquid. Avoid prolonged incubation in wash buffer when performing wash steps.

When making additions to the plate, be careful to avoid damaging the coating, for example by scratching the bottoms or the sides of the wells. One technique to avoid this is to make additions (for a right-handed person) from left to right across the plate, supporting the pipette tips on the right edge of the well with each addition and thus avoiding contact with the bottom or sides of the wells.

During the incubation times, the plate should be covered to minimize evaporation from the wells. This can be done with the adhesive covers provided or by stacking an empty plate on top.

After the last wash step and prior to adding the TMB substrate, wipe the bottom of the plate with a clean paper towel to ensure that moisture or fingerprints do not interfere with the OD reading.

Once the TMB substrate is added it will be converted by the captured HRP to a blue colored product. Generally we find that a 10 to 15 minutes incubation is sufficient for enough color development to discern differences between the standards and the reaction should be stopped at this point. Bear in mind that, given sufficient time, even a small amount HRP is capable of converting all the TMB to product and if this happens it will be difficult to discern differences between differing concentrations of HRP. Keeping OD₄₅₀ values well below 2.0 will result in greatest accuracy as at high absorbance values very little light is reaching the detector and measurements are error prone. (Remember that at an OD of 1.0 only 10% of the light is being detected and at an OD of 2.0 only 1% of the light is reaching the detector).

Assay Protocol

1. Use the plate layout sheet on the back page to plan sample layout on plate and also aid in proper sample and standard identification after the assay. We recommend that assays are carried out in duplicate or (preferably) triplicate in order to minimize spurious results.
2. Dilute the 10x PBS-T and 5x Dilution buffer with water to 1x-strength. Check both concentrate bottles for precipitates before proceeding and if found warm slightly in a water bath to dissolve before proceeding. The 30 ml of 10xPBS-T should be diluted to 300 ml with 270 ml water and the 15 ml of 5x Dilution Buffer should be diluted to 75 ml with 60 ml water.
3. Sample Preparation:
 - A. Prepare test Samples: dilute test sample to 0.5 mg/mL protein concentration with PBS-T (**Please note that the protein samples need to be diluted by PBS-T instead of the dilution solution**). Based on the quantitation limit requirement, spike a certain levels of Protein A standard into the 0.5 mg/ml protein sample for recovery testing. It is recommended to spike 125 µl of the 8 ng/ml Protein A standard into 875 µl of the protein sample (**please make sure the final protein concentration is 0.5 mg/ml and the total volume is 1 ml, smaller volume may impact protein precipitation and spike recovery**) for a quantitation limit (QL) of 2 ng/mg (2 ppm, 1 ng/0.5 mg), the customer can chose to spike higher or lower levels of Protein A based on their QL requirement.
 - B. Denature and remove IgG from control (spiked) and test samples: place the samples in boiling water (about 95°C to 100°C) for 3.5 to 4.5 minutes, target is 4 minutes.
 - a. Allow tubes to cool at room temperature for at least 15 minutes, up to 2 hours
 - b. Centrifuge at 10,000 to 12,000 rpm for 1 to 2 minute to pellet protein, take the supernatant to perform the Protein A analysis.
4. Dilute your sample in 1x dilution buffer; for best accuracy perform serial dilutions over a wide range such that multiple dilutions will span the range of 0.125 – 8 ng /ml. Pipette 100 µL of samples or standards into wells in the plate. Leave several wells empty for background binding determination. Cover plate and incubate 1.5 hour at room temperature.
5. During the above incubation, dilute the 0.5 mg/ml reporting antibody to 5 µg/ml by adding the entire 150 µL to 15 ml of 1x Dilution Buffer.

6. Wash plate by emptying contents and adding 250 μ L of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.
7. Pipette 100 μ L of Reporting Antibody into each well. Cover plate and incubate plate 45 min. at room temperature.
8. During the above incubation, dilute the 4 μ g/ml Streptavidin-HRP conjugate to 0.1 μ g/ml by adding the entire 375 μ L to 15 ml of 1x Dilution Buffer.
9. Wash plate by emptying contents and adding 250 μ L of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.
10. Pipette 100 μ L of Streptavidin-HRP conjugate into wells. Cover plate and incubate plate 30 min. hour at room temperature.
11. Wash plate by emptying contents and adding 250 μ L of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.
12. Add 100 μ L of TMB substrate to each well. Monitor color development and stop reaction by adding 100 μ L of Stop Solution to each well when color development within standards is sufficient. Generally 10-15 minutes time will be sufficient.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm, use three wells without sample as blank such as H10-H12.
14. Either graph the results on log graph paper or use the plate reader's built-in 4-parameter fit software capabilities to calculate Protein A concentration for each sample.

Calculation of Results

Average the triplicate OD readings for each standard, sample and background wells to give a mean OD reading. Subtract the averaged background values from the mean OD values to give a net OD value and create a standard curve using either log graph paper or 4-parameter fit software. Match OD values for the unknowns to [Protein A] using the standard curve, remembering to correct for dilution.

Typical data

Sample	Mean OD	[Protein A] pg/ml
NSB (Buffer blank)	0.1945	0
11 pg/ml Protein A	0.1945	11
34pg/ml Protein A	0.2128	34
103 pg/ml Protein A	0.2699	103
309 pg/ml Protein A	0.4461	309
926 pg/ml Protein A	1.0399	926
2779 pg/ml Protein A	2.2379	2779
8333 pg/ml Protein A	2.8565	8333
25 ng/ml Protein A	3.0933	25000
Unknown 1		
Unknown 2		

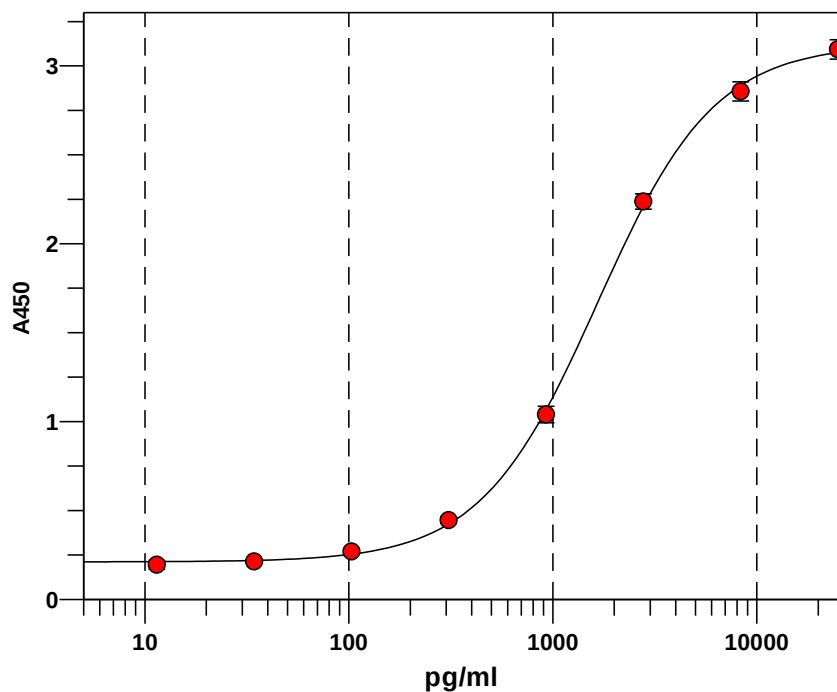


Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												