

Version 1f Last updated 12 March 2026

ab273154

Protein A Detection

Elisa Kit

To estimate the contamination by protein A from *Staphylococcus aureus* in a solution of antibodies.

This product is for research use only and is not intended for diagnostic use.

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Technical Support

1. Overview

The Protein A Detection Elisa Kit (ab273154) provides a quick and simple method to estimate the contamination by protein A from *Staphylococcus aureus* in a solution of antibodies.

The kit includes ready-to-use reagents necessary to analyze 89 samples in 80 minutes.

Capture antibodies coated on the wells, bind the protein A present in the sample and form complexes that are revealed by an anti-Protein A peroxidase conjugated detector antibody. After washing to remove any non-specific binding, the ready-to-use substrate solution is added to microwells and color develops proportionally to the amount of protein A in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

2. Protocol Summary

Prepare all reagents and samples as instructed



Add 100 μL of standards/sample to each well of the strip.



Incubate for 30 minutes at RT, wash the plate 3 times with 300 μL wash buffer.



Add 100 μL of Detection antibody



Incubate for 30 minutes at RT, wash the plate 3 times with 300 μL wash buffer.



Add 100 μL of TMB substrate to each well.



After 10 minutes add 100 μL Stop solution



Results can be directly seen or read at 450nm and 620nm.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website <https://www.abcam.com/en-us>.

4. Storage and Stability

Store kit at 4°C immediately upon receipt. Kit has a storage time of 12 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	1 x96 tests	10 X 96 tests	Storage Condition
Pre-coated microwells strips	6 strips of 16 microwells	60 strips of 16 microwells	+4°C
Sample Diluent	30 mL	500 mL	+4°C
Detection antibody	12 mL	120 mL	+4°C
TMB Substrate	12 mL	120mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
Protein A Dissociation Buffer	12 mL	120 mL	+4°C
Protien A Standards	7 x 0.6 mL	7 x 5 mL	+4°C

Note: This ELISA kit will soon contain the “Easy View” colored reagents. The Standard diluent buffer will now be red, and the Streptavidin-HRP Diluent will be green. Please note that while stock lasts you may still receive colorless diluents. This change does not impact the results provided by the kit or the assay procedure.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- ELISA plate washer
- Wash solution (H₂O, 0.05% Tween 20)
- Standard microplate reader - capable of reading absorbance at 405 nm and 620nm.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample and reagent.
- Pipette samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (25°C or 37°C) prior to use. The kit contains enough reagents for 100 assays.

All reagents are supplied ready to use.

10. Sample Preparation

Make a range of dilutions of the antibody to be analyzed in the sample diluent.

Add one volume of the dissociation buffer to the diluted antibodies and incubate for 5 minutes at room temperature.

11. Assay Procedure

- Equilibrate all prepared reagents to desired assay temperature prior to use.

- 11.1 Transfer 100 μL of standards and diluted samples in each well. Incubate for 30 minutes at room temperature and wash the plate 3 times with 300 μL of washing solution.
- 11.2 Immediately add 100 μL of Detection antibody to each well..
- 11.3 Incubate at room temperature for 30 minutes.
- 11.4 After incubation, remove the solution and wash the wells three times with 300 μL of wash solution.
- 11.5 Add 100 μL of TMB substrate to each well. Tap plate briefly to mix. Incubate for 10 minutes at room temperature.
- 11.6 After incubation add 100 μL of stop solution to each well.
- 11.7 Results can be seen directly or read the absorbance with a microplate reader at 450 nm and 620 nm.

12. Calculations

Standard curve: plot the average value (absorbance 450-620nm) of each standard on the Y axis against their corresponding concentration on the X axis.

12.1. Calculate the average absorbance values for each standard.

12.2. Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding standard concentration on the horizontal axis.

12.3. The protein A concentration in the sample can be calculated by interpolation between standards points on the curve.

13. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

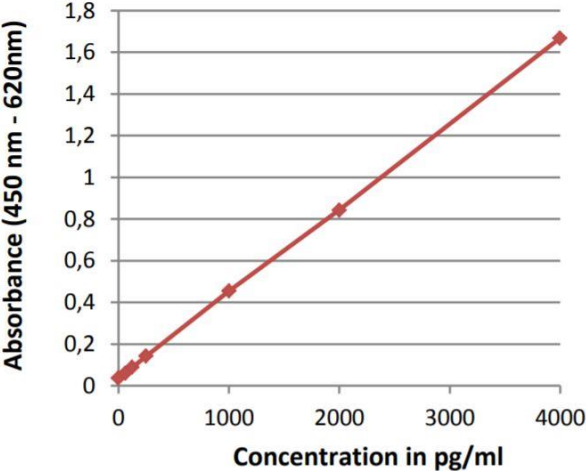


Figure 1. Example of Rat IgG quantitative kit Standard curve

14. Notes

Technical Support

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