

## Protein A Easy ELISA Kit

**Catalog No:** IS006

**Quantity:** 1 x 96 tests

The Protein A ELISA kit provides a quick and simple method to estimate the contamination by protein A from *Staphylococcus aureus* in a solution of antibodies.

Ready-to-use reagents are sufficient for the analysis of 89 samples in 80 min. The kit includes colored buffers to facilitate and control distribution of samples in wells.

### Principle of the Assay:

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

### Specificity:

**Cross reactions:** no cross reaction observed with antibodies

**Matrix effect:** the sample buffer, in general, has no effect on the assay. It is nevertheless recommended to test the effect of the buffer.

**Hook Effect:** no hook effect observed.

### Sensitivity:

The detection range is from 64 pg/ml to 4,000 pg/ml.

### Kit Contents (for 1 x 96 tests):

Item	Description	Quantity
IS006-P	Pre-coated microplates: 96 microwells coated with anti-Protein A polyclonal antibodies	6 strips of 16 wells (2 wells x 8 wells)
IS006-Sd	Protein A standards ( <b>Blue solution</b> ) Concentrations: 0 – 62 – 125 – 500 – 1000 – 2000 - 4000 pg/ml	7 x 0.3 ml
IS006-D	Sample Diluent ( <b>Blue solution</b> )	30 ml
IS006-DB	Protein A Dissociation Buffer (colorless solution)	12 ml
IS006-C	Detection antibody: Peroxidase conjugated anti-Protein A antibody ( <b>Red solution</b> )	12 ml
IS006-T	Substrate solution (TMB)	12 ml
IS006-St	Stop solution (2M HCl)	12 ml

All the kit components are ready-to-use.

### Storage:

All kit components are stable for 12 months when stored at 2-8°C. **Do not freeze.** After opening, reagents must be handled with care to avoid contamination and should be used within 2 months.



## Additional Materials Required:

- Pipettes and tips (20-200 µl)
- ELISA plate washer (recommended)
- Microplate reader for absorbance measurements at 450 nm and 620 nm
- Wash solution: H<sub>2</sub>O, 0.05% Tween 20

Note: Other wash solutions may be used, but they have to be tested with the method.

## Sample Preparation and Storage:

Make a range of dilutions of the antibody to be analyzed in the sample diluent (IS006-D). Then, add one volume of the dissociation buffer (IS006-DB) to the diluted antibodies and incubate for 5 minutes at room temperature.

## Assay procedure:

All steps must be performed at room temperature (RT). Bring all the reagents to room temperature 30 min before use.

Step 1	Distribute 100 µl of standards and samples <b>colored in blue</b> in each well. It is recommended to treat the samples in duplicate.
Step 2	After incubation for 30 minutes at room temperature, wash the plate 3 times with <b>300 µl</b> of washing solution.
Step 3	Add 100 µl of peroxidase conjugated anti-protein A antibody ( <b>red solution</b> ) to each well.
Step 4	After incubation for 30 minutes at room temperature, wash the plate 3 times with <b>300 µl</b> of washing solution.
Step 5	Pipette 100 µl of TMB substrate in each well. Incubate for 10 minutes at room temperature.
Step 6	Stop the reaction by pipetting 100 µl of STOP solution in the same order as for the TMB distribution.
Step 7	Read the absorbance at 450 nm and 620 nm with a microplate reader.

## Calculation of Results:

**Standard curve:** Plot the average value (absorbance 450-620) of each standard on the Y axis against their corresponding concentration on the X axis. Calculate the concentration of Protein A in the sample from the curve (linear regression).

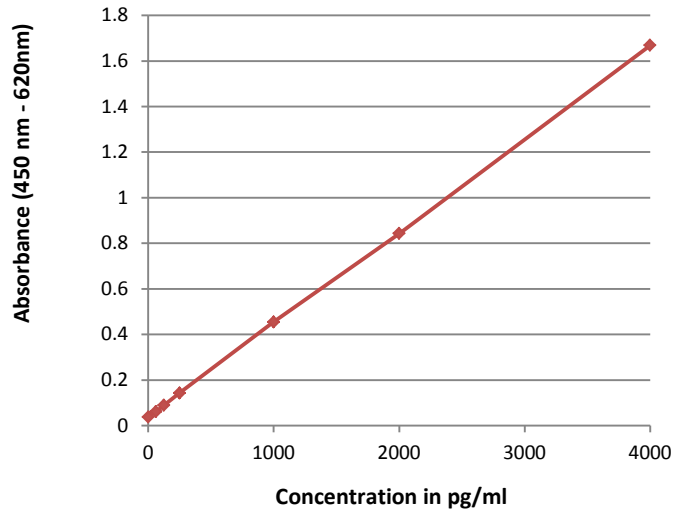
To estimate the rate of contamination of an antibody with protein A, the concentration of Protein A obtained must be reported (in ppm) to the concentration of the antibody.

Example: the concentration of protein A is detected at 800 pg/ml in an antibody with a concentration of 1 mg/ml, therefore the antibody is contaminated with 0.8 ppm Protein A.



**Typical Data:**

This standard curve is shown as an example only. A new standard curve should be performed for each series of samples to be tested.



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