PROTEIN A ELISA KIT

FastELISA®

REF RDB3260

APPLICATION

The RD-Biotech 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.

THRESHOLD DETECTION

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

CONSERVATION

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.

KIT CONTENT

References	Component	Quantity
RDB3260-P	Pre-coated microplates: 96 microwells coated with anti- protein A polyclonal antibody	12 strips of 8 wells
RDB3260-Sd	Protein A standards, concentrations in pg/ml: 0-62-125-500-1000-2000-4000 (Blue solution)	7 x 0,3 ml
RDB3260-D	Sample Diluent (Blue solution)	30 ml
RDB3260-DB	Protein A Dissociation Buffer (colorless solution)	12 ml
RDB3260-C	Conjugated antibody: Peroxydase conjugated Anti-Protein A antibody (Red solution)	12 ml
RDB3260-T	TMB substrate	12 ml
RDB3260-St	Stop solution (HCl 2M)	12 ml

ADDITIONAL MATERIAL 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_2O, 0.05% Tween 20. Other wash solutions may be used but they have to be tested with the method.

SAMPLE PREPARATION

Make a range of dilutions of the antibody to be analyzed in the sample diluent (RDB-3260-D). Then, add one volume of the dissociation buffer (RDB-3260-DB) to the diluted antibodies and incubate for 5 minutes at room temperature.

ASSAY PROCEDURE

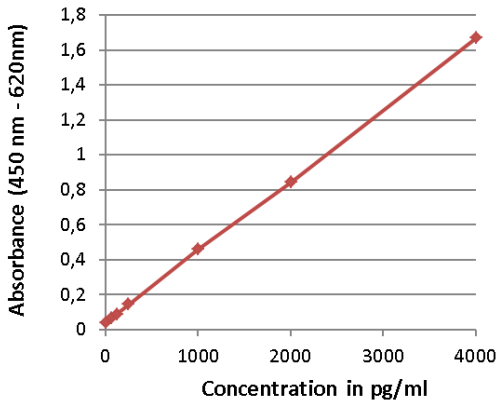
All steps must be performed at room temperature (RT). Bring all reagents at RT for 30 min before use.

STEP 1	Distribute 100µl of standards and samples colored in blue 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 300 µl of washing solution.
STEP 3	Add 100 µl of peroxydase conjugated anti-protein A antibody (red solution) to each well.
STEP 4	After incubation for 30 minutes at room temperature, wash the plate 3 times with 300 µl of washing solution.
STEP 5	Add 100 µl of TMB substrate solution in each well. Incubate for 10 minutes at room temperature.
STEP 6	Stop the reaction with 100 µl of STOP solution.
STEP 7	Read the absorbance at 450 nm and 620 nm with a microplate reader

RESULTS INTERPRETATION AND VALIDATION

Standard curve: plot the average value (absorbance 450-620nm) 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.

EXAMPLE OF STANDARD CURVE



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