APPLICATION

The Protein A quantification kit provides a rapid and easy method **(one step ELISA)** 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 up to **89 or 445 samples** in **80 min** (for RDB-3260-1 or RDB-3260-5 respectively).
Buffer solutions are color coded in order to simplify pipetting steps.

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 AND SENSIVITY

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.
The detection range is from **64 pg/ml to 4000 pg/ml**.

STORAGE

Storage at 2-8°C. After opening, reagents must be handled with care to avoid contamination and should be used within 2 months. Do not freeze.

KIT CONTENTS

Code	Component	Quantity 1x96 wells RDB-3260-1	Quantity 5x96 wells RDB-3260-5
RDB3260-P	Pre-coated microplates: anti-protein A polyclonal antibodies	12 strips of 8 wells	60 strips of 8 wells
RDB3260-Sd	Protein A standards (**Blue solution**) Concentrations (pg/ml): 0 - 62 - 125 - 500 - 1000 – 2000 - 4000	7 x 0.3 ml	7 x 0.5 ml
AS31	Sample diluent (**Blue solution**)	30 ml	150 ml
RDB3260-DB	Protein A dissociation buffer (Colorless solution)	12 ml	60 ml
RDB3260-C	Detection antibody (**Red solution**)	12 ml	60 ml
AS29	TMB	12 ml	60 ml
AS40	Stop solution	12 ml	60 ml

ADDITIONNEL MATERIEL REQUIRED

Pipettes (20-200 µl).

ELISA plate washer (recommended).

Microplate reader for absorbance measurements at 450 nm and at 620 nm.

Wash solution: H2O + 0.05% Tween 20 or PBS 1X 0.05% Tween 20.

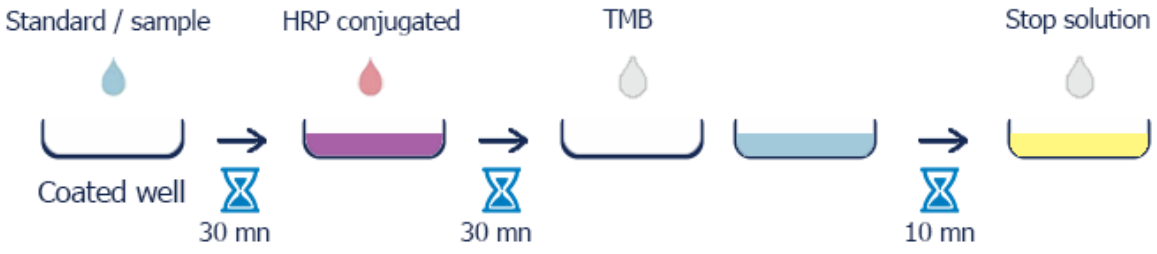
SAMPLE PREPARATION AND STORAGE

Make a range of dilutions of the antibody to be analyzed in the sample diluent (RDB-3260-D). Then, samples are diluted volume to volume in the dissociation buffer (RDB-3260-DB) 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, remove the solution and 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. Mix gently until obtaining a homogeneous **purple color**. Incubate the plate for **30 min** at RT.
STEP 4	After incubation, remove the solution and wash the plate three times each with 300 µl of the wash solution.
STEP 5	Pipette 100 µl of TMB substrate into each well. Incubate the plate for **10 min** at RT.
STEP 6	Stop the reaction by pipetting 100 µl of STOP solution in the same order as for TMB distribution.
STEP 7	Read the absorbance at **450 nm** and 620 nm with a microplate reader.



RD-Biotech
www.rd-biotech.com
RD-Biotech S.A.S.
Technopole TEMIS Santé
18, rue Françoise Dolto
25000 Besançon - France
ISO 9001:2015 certification
RESEARCH TAX CREDIT
APE 7211Z
Tél: +33 (0)3 81 53 88 37
Fax: +33 (0)3 81 53 89 65
Email: info@rd-biotech.com
SIREN: 442 804 266
TVA: FR 41442804266
A company of the group
mAbxperts
www.mabexperts.com

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INTERPRETATION AND VALIDATION OF RESULTS

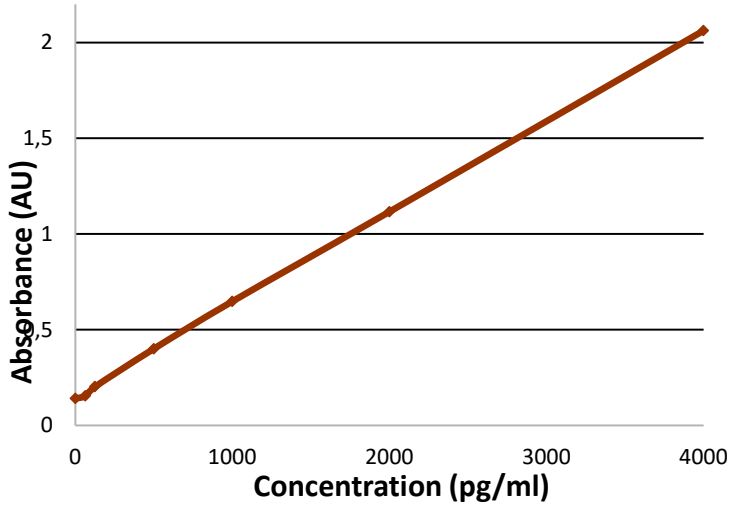
Standard curve: plot the average value (absorbance 450-620nm) of each standard on the Y axis against their corresponding concentration on the X axis.

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.

Typical curve:



Concentration (pg/ml)	A450nm-A620nm
0	0.141
63	0.154
125	0.202
500	0.401
1000	0.648
2000	1.117
4000	2.063