

Kit manual of

Protein A ELISA

for samples without IgG

Catalogue Number: 03-96

Edition: 010508

IMMUNSYSTEM AB

S-751 83 Uppsala, Sweden

Phone +46 18 53 89 09

Fax +46 18 53 89 97

GENERAL INFORMATION

See Kit manual of Protein A ELISA for samples containing IgG

TEST PRINCIPLE

This ELISA kit is designed to detect protein A in solutions without IgG. The assay is a sandwich ELISA, utilising microtitre strips coated with affinity-purified chicken anti-protein A, which will bind protein A. Unbound material are removed by washing. Bound protein A is detected by biotinylated chicken anti-protein A. Unbound conjugate is washed away, and a streptavidin horseradish peroxidase conjugate is added. After

washing, a substrate reactive with horseradish peroxidase is added. Colour development is due to conversion of the substrate by the conjugate. A positive result is indicated as a colour change. The colour can be read visually or by a microplate photometer at 450 nm. In order for the test to work properly, all samples are neutralised with a dilution buffer.

THE EFFECT OF SAMPLE PREPARATION ON RECOVERY OF SPA

See Kit manual of Protein A ELISA for samples containing IgG

VALIDITY, SENSITIVITY AND PRECISION

To ensure validity of this assay the standard reference E (0,4 ng/ml) should have a corrected absorbance value greater than 0,1. This assay will yield a positive result if

the concentration of protein A in the sample is equal to or exceeds **0,1 ng/ml**. Coefficients of variation for duplicate measurements are typically < 10%.

MATERIALS NEEDED BUT NOT PROVIDED

1. Precision pipettes. 10 to 1.000 µl
2. Humid chamber
3. Microplate photometer
4. Disposable pipette tips
5. Eppendorf tubes
6. Wash bottle
7. Distilled water

CONTENTS

See Kit manual of Protein A ELISA for samples containing IgG

PRECAUTIONS

See Kit manual of Protein A ELISA for samples containing IgG

PREPARATION OF REAGENTS

See Kit manual of Protein A ELISA for samples containing IgG

SAMPLE PREPARATION

See Kit manual of Protein A ELISA for samples containing IgG

TEST PROCEDURE

All reagents should equilibrate to room temperature +18°C to +25°C (64 to 77°F) before use.

1. Preparation of standard references:

1.1. Label 6 Eppendorf tubes from A to F for each set of standard references. Add 990 µl of PBS-Tween buffer to tube A and B and add 500 µl of PBS-Tween buffer to tube C-F (see table 1, below).

1.2. Add 10 µl protein A reference (0.5 mg/ml) and dilute the standard reference A with PBS-Tween buffer and mix well on vortex.

1.3. Transfer 10 µl of standard reference A to tube B and mix well on vortex. Prepare standard references 'C' to 'E' according to table 1 (see below).

Table 1

Standard reference	Source solution	PBS-Tween buffer	Protein A conc. (ng/ml)
A	10 µl of Protein A ref.	990 µl	5.000
B	10 µl of reference A	990 µl	50
C	125 µl of reference B	500 µl	10
D	125 µl of reference C	500 µl	2
E	125 µl of reference D	500 µl	0,4
F	None	500 µl	0

2. Preparation of acid samples:

1. Add 1/10 of the volume of **Neutratlisation buffer** to each sample collection tube (see Sample Preparation) before the acid samples is added. Dilution factor: 0,9. Calculation of dilution factor (DF) for each sample: additional dilutions done except the Neutralisation, is multiplied with 0,9. Note the final DF for each sample in table 3.
2. **If your samples are neutral**, go to step 4 (Do not forget to note their dilution factor).

3. Add 100 µl of the **standard references B, C, D, E, and F** to appropriate wells. It is recommended to test standard references in duplicate.

4. Add 100 µl of the **neutralised samples and sample buffer** (with no protein A) to the appropriate wells. For confirmation purposes it is recommended to run a duplicate for each test sample.

TEST PROCEDURE (Continue)

5. **Incubate** the microtitre wells with samples and standard references 60 minutes at room temperature +18°C to +25°C (64 to 77°F) in a humid chamber or with the wells sealed with tape.

6. Empty wells and **rinse** 4 times with PBS-Tween buffer, filling all wells to the top for each rinse. Empty all fluid from the wells and tap the plate hard to remove the last traces of fluid.

7. Add 100 µl of diluted **Biotinylated anti-protein A** to each well. Incubate for 60 minutes at room temperature +18°C to +25°C (64 to 77°F) in a humid chamber or with the wells sealed with tape.

8. Wash the strips (see step # 6). Add 100 µl of diluted **HRP conjugate** to each well. Incubate for 30 minutes at

room temperature +18°C to +25°C (64 to 77°F) in a humid chamber or with the wells sealed.

9. Wash the strips (see step # 6). Add 100 µl of **Substrate solution** to each well and incubate 10 minutes at room temperature +18°C to +25°C (64 to 77°F) in darkness. Begin timing after the first well is filled.

10. Stop the reaction at 10 minutes by adding 100 µl **Stop solution** to each well. Add the Stop solution in the same order as the Substrate solution was added in step # 9.

11. **Measure** the absorbance of the samples and standard references at 450 nm within 30 minutes after adding the stop reagent (use air as blank).

12. **Calculate** the mean corrected absorbance values for each unknown test sample and standard references.

INTERPRETATION OF THE RESULTS

See Kit manual of Protein A ELISA for samples containing IgG

CALCULATIONS OF PROTEIN A IN THE SAMPLES

See Kit manual of Protein A ELISA for samples containing IgG

REFERENCES

See Kit manual of Protein A ELISA for samples containing IgG