Instructions for Use
Life Science Kits & Assays

PRRSV CHECK ELISA

analytikjena
An Endress+Hauser Company

Order No.:
847-0104000120 96 reactions
847-0104480120 480 reactions

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Manufacturer:
AJ Roboscreen GmbH
Hohmannstraße 7
04129 Leipzig
Made in Germany!

Phone +49 341 989734 0
Fax +49 341 989734 199

Distribution/Publisher:
Analytik Jena AG
Konrad-Zuse-Straße 1
07745 Jena · Germany

www.analytik-jena.com
info@analytik-jena.com

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1 Introduction

1.1 Intended use

The porcine reproductive and respiratory syndrome virus (PRRSV) poses one of the most serious threats to the herd health status and the profitability of pig farms connected with it. In order to combat these risks, PRRSV CHECK ELISA represents a new test generation for screening, thanks to its particularly simple and reliable performance. PRRSV CHECK ELISA is an immunological detection method to determine antibodies against PRRSV in serum (including vaccination serum). Samples proven to be positive may undergo a differentiation in PRRSV type-1 and/or type-2, using the corresponding PRRSV NA/EU TYP ELISA (order number: 847-0104000121).

1.2 Warranty and technical support

The manufacturer guarantees the correct functioning of the kit for the applications described in the instructions for use (IFU). During the warranty period, PRRSV CHECK ELISA allows for precise and reproducible data collection in connection with excellent sensitivity. Any warranty claims shall only be valid if the general principles of Good Laboratory Practice (GLP) and the manufacturer's recommendations are observed.

To improve the application and design, Analytik Jena AG reserves the right of product replacement or modification. The manufacturer may be contacted at any time for questions and problems or technical support concerning the detection of PRRSV.

CONSULT INSTRUCTION FOR USE

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.3 Notes on the use of this instructions for use

For easy reference and orientation, the IFU uses the following warning and information symbols as well as the shown methodology:

REF	**REF** Catalogue number
Σ N	**Content** Contains sufficient reagents for <N> tests
30 °C 4 °C	**Storage conditions**
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiration date
	Manufactured by
	For single use only
	Note / Attention Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following abbreviations are used in the IFU:

ELISA	Enzyme-linked immunosorbent assay
EU	Europe
GLP	Good Laboratory Practice
HRP	Horseradish peroxidase
IFU	Instruction for use
NA	North America
OD	Optical density
PRRSV	Porcine reproductive and respiratory syndrome virus
RT	Room temperature (15 - 25°C)
TMB	Tetramethylbenzidine

2 Safety precautions

NOTE
We recommend reading this chapter thoroughly before using this kit, to ensure the safety of the user and error-free utilization.
Any safety instructions and additional information of this IFU must be observed at all times.

Read and make sure you understand the operating instructions completely and thoroughly before carrying out the test. Use the currently valid version from the kit.

Notify the respective supplier in writing within one week from receiving the merchandise, should the test pack be substantially damaged. Damaged components must not be used to carry out the test, however, they should be kept until the transport damages are finally settled.

Comply with Good Laboratory Practice and safety regulations. Wear laboratory coats, disposable Latex gloves and safety goggles whenever the need arises.

Reagents of this kit which contain hazardous substances may cause irritations to the eyes and skin. See indications under COMPONENTS OF THE KIT and on the labels. Safety data sheets of this product are available upon request.

Chemicals and prepared or used reagents shall be disposed of as hazardous waste in compliance with the respective national regulations.

The cleaning staff has to be instructed by the experts with regard to any potential risks and the appropriate handling of such substances.

Avoid any contact with stopping solution. This may cause irritations to the skin and chemical burns.

FOR SINGLE USE ONLY!
This kit is made for single use only!

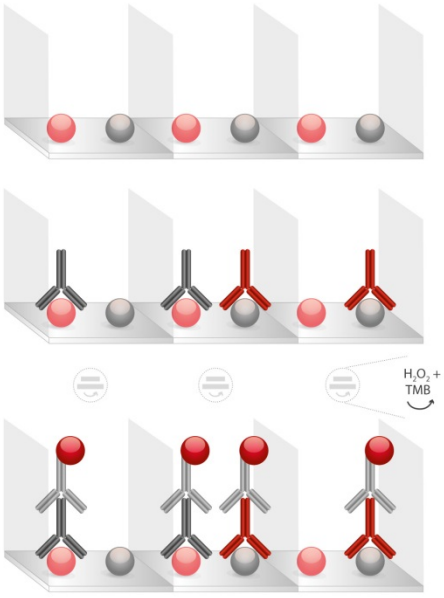
ATTENTION!
Don’t eat or drink components of the kit!
The kit shall only be handled by educated personnel in a laboratory environment!

3 Test principle

PRRSV CHECK ELISA is based on a direct ELISA and is suitable to demonstrate the presence of antibodies against PRRS viruses in biological samples from pigs, e.g. serum and saliva. The PRRSV antigens bound to the plate, a mixture of virus antigens of the EU and/or NA type PRRSV strains are recognized and bound by anti-PRRSV antibody in serum samples of pigs.

A positive and a negative control are applied as well, for repeat determination, and serve the evaluation of test quality as well as the classification of the measured values of the tested pig samples.

The bound antibodies are determined by means of HRP-conjugated anti-pig antibody. The detection of the bound HRP-antibody-antigen complex is visualized by means of TMB/H_2O_2 staining.



1. **Ready for use: Microtitre plate** coated with PRRSV antigens for NA and EU virus type

2. **Binding of anti-PRSSV antibodies** after incubation with sample

3. **Direct detection by means of HRP-conjugated anti-pig antibody (TMB substrate)**

4 Performance assessment

120 serum samples were examined for the clinical validation of PRRSV CHECK ELISA. The extraordinary stability of the kit allows for a simple data analysis by means of relative reaction values (see data analysis).

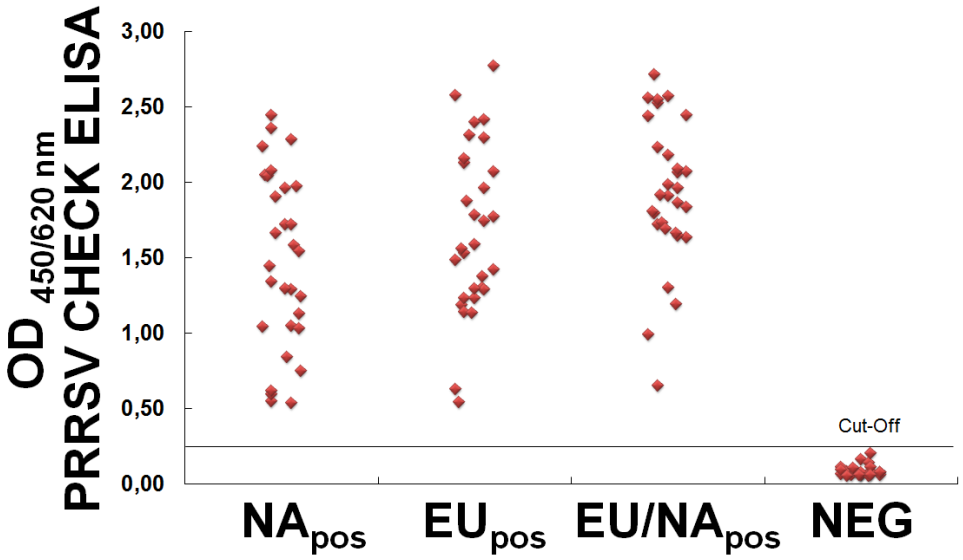


Figure 1: Measurements of samples of positive (n=90) as well as negative sera (n=30). Sensitivity as well as a specificity of 100 % were determined.

5 Kit components, storage and stability

PRRSV CHECK ELISA is available in following two formats:

- Cat.-no.: 847-0104000120 for 96 reactions
- Cat.-no.: 847-0104480120 for 480 reactions

Kit components for 96 reactions

Component	Σ	Description
D1 Immunostrip	1	**Bag** containing **12 x 8** coated immunostrips.
D2 10X Wash buffer	1	**100 ml** 10fold concentrated wash buffer containing TRIS, detergents and sodium merthiolate.
D3 Positive control	3	Lyophilized anti-PRRSV antibodies for test verification containing Proclin 300.
D4 Negative control	3	Lyophilized pig control serum w/o PRRSV antibodies for test verification containing Proclin 300.
D5 10X HRP anti-pig-IgG	1	**2 ml** Horseradish peroxidase conjugated anti-pig antibody containing Proclin 300.
D6 Assay buffer	1	**50 ml** Assay buffer containing buffered sodium chloride solution with BSA, detergents and Proclin 300.
D9 Staining solution	1	**20 ml** ready-for-use TMB/peroxide solution.
D10 Stopping solution	1	**25 ml** 1 M sulphuric acid.
Sealing tape	2	
Instructions for use	1	

Kit components for 480 reactions

Component	Σ	Description
D1 Immunostrip	5	**Bags** containing each **12 x 8** coated immunostrips.
D2 10X Wash buffer	1	**200 ml** 10fold concentrated wash buffer containing TRIS, detergents and sodium merthiolate.
D3 Positive control 	10	Lyophilized anti-PRRSV antibodies for test verification containing Proclin 300.
D4 Negative control 	10	Lyophilized pig control serum w/o PRRSV antibodies for test verification containing Proclin 300.
D5 10X HRP anti-pig-IgG 	1	**10 ml** Horseradish peroxidase conjugated anti-pig antibody containing Proclin 300.
D6 Assay buffer	1	**200 ml** Assay buffer containing buffered sodium chloride solution with BSA, detergents and Proclin 300.
D9 Staining solution	1	**75 ml** ready-for-use TMB/peroxide solution.
D10 Stopping solution	1	**100 ml** 1 M sulphuric acid.
Sealing tape	10	
Instructions for use	1	

6 Component preparation

6.1 1X Wash buffer solution

Dilute 10X Wash buffer **D2** using bi-distilled water before the first wash step of the immunoassay.

Volume of 1X Wash buffer solution	Volume of 10X Wash buffer D2	Volume of de-ionized or bi-distilled water
300 ml	30 ml	270 ml
400 ml	40 ml	360 ml
500 ml	50 ml	450 ml
1000 ml	100 ml	900 ml
1500 ml	150 ml	1350 ml
2000 ml	200 ml	1800 ml

6.2 Positive control D3

To test the **serum samples**, add 1 ml of assay buffer **D6** for positive control **D3** and mix quickly, e. g. within 2 s by vortex. Build aliquots subsequently.

For **saliva** testing control **D3** has to be diluted **1:15** using assay buffer **D6** – e. g. 0.1 ml solved **D3** and 1.4 ml **D6**.

6.3 Negative control D4

To test the **serum samples**, add 1 ml of assay buffer **D6** for negative control **D4** and mix quickly, e. g. within 2 s by vortex. Build aliquots subsequently.

For **saliva** testing control **D4** has to be diluted **1:15** using assay buffer **D6** – e. g. 0.1 ml solved **D4** and 1.4 ml **D6**.

6.4 1X HRP anti-pig-antibody

Dilute **10X HRP anti-pig antibody D5** at a 1:10 ratio with assay buffer **D6** and mix it by shaking the tubes or bottle.

Number of immuno strips	Volume of 10X HRP D5	Volume of assay buffer D6
1 - 4	0.4 ml	3.6 ml
5 - 8	0.8 ml	7.2 ml
9 - 12	1.2 ml	10.8 ml
24	2.5 ml	22.5 ml
36	4.0 ml	36.0 ml
48	5.0 ml	45.0 ml
60	7.0 ml	63.0 ml

7 Storage and expiry date

The kit is delivered at ambient temperature and should be stored at 6 ± 4 °C. Protect from heat and direct sunlight. Under these conditions, the kit has a life time as indicated on the kit box while retaining its endurance and stability.

Prepared kit components have the following expiry dates:

Component	Preparation step	Expiry date
D1	Coated immuno strips after opening of bag, taking of strips out and closing of bag.	At 6 ± 4 °C up to 4 weeks.
D2	1X Ready-to-use washing solution.	At RT up to 1 week.
D3 **D4**	Controls D3 and D4 dissolved in D6, aliquot.	Up to 4 x freezing/ thawing cycles at -20 ± 5 °C.
D5	1X Ready-to-use HRP-conjugate.	At 6 ± 4 °C up to 4 h.

8 Components not included in the kit

- Pipettes (Multipette Eppendorf or comparable products, < 3 % CV)
  Volumes: 10 - 100 µl; 100 - 1000 µl
- 8-channel micro-pipette with reagent vessels
- Vortex mixer
- Automatic or semi-automatic wash system for microtiter plates
- Bi-distilled water or de-ionized water
- Pipette tips, stop-watch
- Measuring equipment for microtiter plates to measure the absorption at 450/620 nm
- Tubes to dilute samples (disposable polypropylene tubes)

9 ELISA procedure

9.1 Sample preparation

- The samples to be tested should be heated to be at room temperature
- Mix the samples for a short moment before testing, e. g. by vortexing for 6 - 10 s
- Pre-dilute **serum samples** at ratio of **1:50** by means of assay buffer **D6**
- **Saliva samples** are used at dilution of **1:2** using assay buffer **D6**

9.2 Test conditions

- Controls **D3** and **D4** should be prepared respectively before applying the samples.
- For **saliva** testing controls **D3** and **D4** have to be diluted **1:15** using assay buffer **D6**
- Application diagram for 20 serum samples as an example:

D3	Sample 5	Sample 13
D3	Sample 6	Sample 14
D4	Sample 7	Sample 15
D4	Sample 8	Sample 16
Sample 1	Sample 9	Sample 17
Sample 2	Sample 10	Sample 18
Sample 3	Sample 11	Sample 19
Sample 4	Sample 12	Sample 20

10 Protocol for serum samples

1. Respectively apply 100 µl of the serum samples to be tested, pre-diluted at a ratio of 1:50.

2. Respectively apply 100 µl of controls **D3** and **D4** for repeat determination in 2 cavities respectively.

NOTE
Replace pipette tips in-between the application of the individual samples and the controls.

3. Seal immuno strips by using sealing tape and incubate for 60 min at RT.

4. Remove sealing tape and wash for 5 times with 300 µl 1X Wash buffer solution, manually or by means of ELISA plate washer.

NOTE
Cautiously remove sealing tape in order to prevent splashes from the cavities.

5. Pipette 100 µl of 1X HRP anti-pig antibody per cavity.

6. Seal immunostrips by using the sealing tape and incubate for 60 min at RT.

7. Remove sealing tape and wash for 5 times with 300 µl 1X Wash buffer solution, manually or by means of ELISA plate washer.

NOTE
After step 7 of the ELISA, the staining should occur right after this process (step 8 and 9).

8. Staining: Pipette 100 µl of staining solution **D9** per cavity and incubate for 30 min at RT in the dark.

9. Terminate staining process by adding 150 µl of Stopping solution **D10** per cavity.

10. Measuring the absorption: Mix the plate for 3 - 5 s by means of a plate shaker of the measuring equipment. After a waiting time of 5 s, the degree of staining should be measured at a wave length of 450 nm against a reference wave length of 620 nm, within 10 min after finishing the staining.

NOTE
In samples with a high concentration of specific antibodies, the dye which forms may be precipitated, due to intensive staining. Therefore mixing the plate and 10 min at maximum of time lapse until the measurement takes place is recommended.

11 Protocol for saliva samples

1. Respectively apply 50 µl of the saliva samples to be tested to each well followed by 50 µl of assay buffer **D6**.

2. Respectively apply 100 µl of **1:15** diluted controls **D3** and **D4** for repeat determination in 2 cavities respectively.

NOTE
Replace pipette tips in-between the application of the individual samples and the controls.

3. Seal immuno strips by using sealing tape and incubate for 18 ± 1 h at 6 ± 4 °C.

4. Remove sealing tape and wash for 5 times with 300 µl 1X Wash buffer solution, manually or by means of ELISA plate washer.

NOTE
Cautiously remove sealing tape in order to prevent splashes from the cavities.

5. Pipette 100 µl of 1X HRP anti-pig antibody per cavity.

6. Seal immuno strips by using the sealing tape and incubate for 60 min at RT.

7. Remove sealing tape and wash for 5 times with 300 µl 1X Wash buffer solution, manually or by means of ELISA plate washer.

NOTE
After step 7 of the ELISA, the staining should occur right after this process (step 8 and 9).

8. Staining: Pipette 100 µl of staining solution **D9** per cavity and incubate for 30 min at RT in the dark.

9. Terminate staining process by adding 150 µl of Stopping solution **D10** per cavity.

10. Measuring the absorption: Mix the plate for 3 - 5 s by means of a plate shaker of the measuring equipment. After a waiting time of 5 s, the degree of staining should be measured at a wave length of 450 nm against a reference wave length of 620 nm, within 10 min after finishing the staining.

NOTE
In samples with a high concentration of specific antibodies, the dye which forms may be precipitated, due to intensive staining. Therefore mixing the plate and 10 min at maximum of time lapse until the measurement takes place is recommended.

12 Data analysis

For serum analysis measured optical density (OD) of positive control **D3** has to be > 0.8, of negative control **D4** < 0.15.

For saliva analysis measured optical density (OD) of positive control **D3** has to be > 0.2, of negative control **D4** < 0.08.

Conversion of sample OD into relative reaction values

The sample ODs are corrected by applying the negative control (NC) ODs (mean value) and converted with reference to the positive control (PC) ODs (mean value).

FORMULA

$$\text{Reaction value} = \frac{OD_{Sample} - OD_{NC}}{OD_{PC} - OD_{NC}}$$

All reaction values **< 0.35** are negative with regard to anti-PRRSV antibody.

All reaction values **≥ 0.35** are positive with regard to anti-PRRSV antibody.

engl. 04/17 – Analytik Jena AG, Jena

Headquarters

Analytik Jena AG
Konrad-Zuse-Str. 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279
info@analytik-jena.com
www.analytik-jena.com

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Subject to changes in design and scope of delivery as well as further technical development!