Instructions for Use
Life Science Kits & Assays

PRRSV NA EU TYP ELISA

analytikjena
An Endress+Hauser Company

Order No.:
847-0104000121 192 reactions

Publication No.: Manual_PRRSV_TYP_e_rev.1

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1 Introduction

1.1 Intended use

The porcine reproductive and respiratory syndrome virus, PRRSV, with pigs poses one of the most serious threats to the herd status and the profitability of pig farms connected with it. In order to combat these risks, PRRSV CHECK ELISA (order number: 847-0104000120) represents a new test generation for proving the contact with the virus, thanks to its particularly simple and reliable performance. Samples proven to be positive may be differentiated by type-specific antibody detection by means of PRRSV NA/EU TYP ELISA.

NOTE

PRRSV NA/EU TYP ELISA is not suitable as a screening test.

Testing PRRSV-antibody negative pig sera may lead to wrong positive reactions. PRRSV CHECK ELISA, catalogue number (847-0104000120), is suitable as a screening test to select the sera to be differentiated.

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 NA/EU TYP 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:

Symbol	Information
	**REF** Catalogue number
	**Content** Contains sufficient reagents for <N> tests
	**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
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!

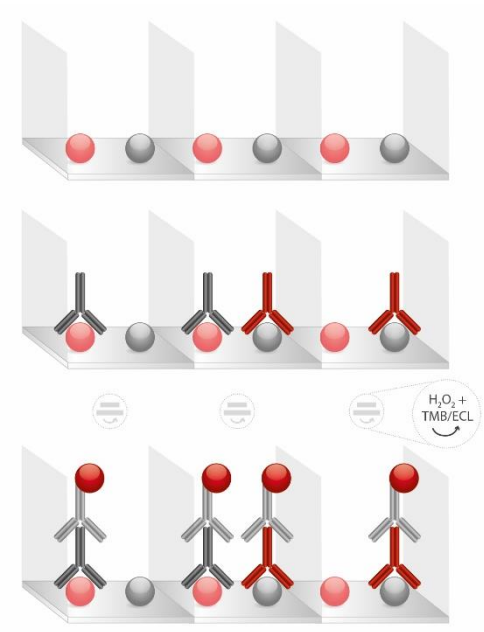
The kit shall only be handled by educated personnel in a laboratory environment!

3 Test principle

PRRSV NA/EU TYP ELISA is based on a direct ELISA. The PRRSV antigens bound to the plate, a mixture of recombinant protein fragments attached to a carrier protein (NSP7, GP5 and GP3), which are specific to the NA and/or EU type respectively, are separately recognised and bound by Anti-PRRSV antibody in serum samples of pigs according to their respective NA and EU type. To match all reactivities, control cavities were coated with non-specific control proteins.

A positive control with specific anti-PRRSV antibody against NA and/or against EU and a negative control without any specific antibody against PRRSV (normal serum) provide for the reconciliation of the measured raw data.

The bound antibodies are determined by means of HRP-conjugated anti-pig-IgG. The detection of the bound HRP-antibody-antigen complex is visualised 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 Kit components, storage and stability

Component	Σ 96	Description
Immunostrip **D1**	2 plates à 12 x 8	Immunostrips, 6 strips/plate coated with PRRSV proteins of NA type (blue marked) and 6 strips/plate with control protein and/or of EU type (red marked) and control protein, blocked, stabilized, ready for use.
10 x wash buffer **D2**	1 x 100 ml	10 x wash buffer contains sodium merthiolate
Positive control **D3.1** 	3	Lyophilized NA type anti-PRRSV antibody to verify the test; contains Proclin 300
Positive control **D3.2** 	3	Lyophilized EU type anti-PRRSV antibody to verify the test; contains Proclin 300
Negative control **D4** 	6	Lyophilized pig IgG without PRRSV antibody to verify the test; contains Proclin 300
10 x HRP anti-pig-IgG **D5** 	2 x 2 ml	Horseradish peroxidase conjugated anti-pig antibody; contains Proclin 300
Dilution buffer **D6**	1 x 60 ml	Dilution buffer contains buffered sodium chloride solution with BSA; contains Proclin 300
Staining solution **D9**	2 x 20 ml	Ready-for-use TMB/peroxide solution
Stopping solution **D10**	2 x 25 ml	1 M sulphuric acid
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5 Component preparation

5.1 1x wash buffer D2

Dilute 10x wash buffer **D2** using distilled water before the first wash step of the immunoassay.

Volume of wash buffer	Volume of 10 x wash buffer D2	Volume of bidest 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

5.2 Positive control D3.1 and/or D3.2

Add 250 µl of dilution buffer **D6** to positive control **D3.1** and/or **D3.2** and vortex for a short moment. Aliquot subsequently.

5.3 Negative control D4

Add 250 µl of dilution buffer D6 to negative control D4 and vortex for a short moment. Aliquot subsequently.

5.4 1x HRP anti-pig-IgG D5

Dilute HRP anti-pig-IgG **D5** at a 1:10 ratio with dilution buffer **D6**. Mix by means of shaking the tubes.

Number of Immunostrips	Volume of 10 x HRP D5	Volume of dilution 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

6 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 of at least 6 months while retaining its endurance and stability. Lot depended shelf life is indicated on the kit label.

Prepared kit components have the following expiry dates:

Component	Preparation step	Expiry date
Immunostrip **D1**	Coated immunostrips after opening the bag, taking the strips out and closing the packaging.	Up to 4 weeks at 6 ± 4°C.
1x wash buffer **D2**	1x Ready-for-use wash solution.	1 week at RT
Positive controls **D3.1 and D3.2** Negative control **D4**	Controls **D3** and **D4** dissolved in D6, aliquot.	4 x freeze at -20 ± 5°C / defreeze cycles
10 x HRP anti-pig-IgG **D5**	Diluted HRP conjugate.	4 hours at 6 ± 4°C

7 Components not included in the kit

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

8 ELISA procedure

8.1 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 dilution buffer **D6**

8.2 Test conditions

- Controls **D3.1**, **D3.2** and **D4** should be prepared respectively before applying the samples.
- Application diagram for ten serum samples (1-10) as an example:

  Blue: Antigen cavity (A) → NA strain

  Red: Antigen cavity (A) → EU strain

  Green: Control cavity (C)

	1	2	3	4	5	6	7	8	9	10	11	12
A	D 3.1	3	7				D 3.1	3	7			
B	D 3.1	3	7				D 3.1	3	7			
C	D 4	4	8				D 4	4	8			
D	D 4	4	8				D 4	4	8			
E	1	5	9				1	5	9			
F	1	5	9				1	5	9			
G	2	6	10				2	6	10			
H	2	6	10				2	6	10			

9 Protocol

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 **D3.1, D3.2** 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 immunostrips by using the sealing film and incubate for 15 min at RT.
4. Remove sealing film and wash for 5 x with 300 µl wash buffer **D2**, manually or by means of ELISA plate washer.

NOTE

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

5. Pipette 100 µl of diluted (1:10) conjugate solution **D5** per cavity.
6. Seal immuno strips by using the sealing film and incubate for 60 min at RT.
7. Remove sealing film and wash for 5 x with 300 µl wash buffer **D2**, 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 10 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 positive control D3 and/or 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 are recommended.

10 Data analysis

The raw data (R) of the optical density determined for antigen (A) of the samples (Sa) and controls (positive control PC and negative control NC) is corrected by applying the OD of the background determined for control protein (C) and therefore results in the measuring value (MV) of the samples and/or controls.

MV_{Sa} Sample measuring value

MV_{NC} Negative control **D4** measuring value

MV_{PC} Positive control **D3.1** and/or **D3.2** measuring value

FORMULA

$$MV_{Sa/PC/NC} = OD\ A_{Sa/PC/NC} - OD\ C_{Sa/PC/NC}$$

The $OD_{450/620}$ nm measuring value of the negative control has to be < 0.5. The $OD_{450/620}$ nm measuring value of the positive controls has to be > 0.5 and < 2.

The MV_{NC} has to be < 0.5.

The MV_{PC} has to be > 0.5 and < 2.

The reaction values and cutoff are to be calculated as shown below.

Samples with a **reaction value ≥ cut-off** are classified as positive with regard to their specific reactivity due to the presence of anti-PRRSV antibody.

Samples with a **reaction value < cut-off** are classified as negative with regard to their specific reactivity due to the presence of anti-PRRSV antibody.

FORMULA

$$\text{Reaction value} = \frac{MV_{Sa} - MV_{NC}}{MV_{PC} - MV_{NC}}$$

$$\text{Cutoff} = 2 \cdot MV_{NC}$$

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