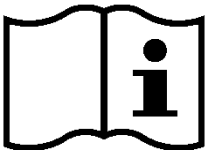

Useŕs Manual

Pseudorabies Virus Antibody ELISA

A monoclonal antibody-mediated ELISA to detect of Pseudo rabies Virus (Aujeszky's disease virus) gE antibodies in serum or plasma samples

REF P1001-AB01

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Gebruik alleen de juiste versie van het protocol dat meegestuurd wordt met de kit.
Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Test kit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

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1. Introduction

In piglets, a variety of neurological signs are associated with the disease, but respiratory signs are often the most striking clinical feature, the disease is less pronounced in older pigs and, after recovery, i.e. in adult pigs, a lifelong latent infection is established. From such asymptotic pigs, ADV has been isolated from cranial ganglia and lymphoid tissue. The virus can be transmitted by physical contact with infected animals or through maternal infection of foetal or suckling pigs by reactivated virus in lately infected sows.
All herds in endemic regions should be monitored for the presence of infection and uninfected herds protected by control measures. Some countries practice vaccination, while some others try to control the spread by culling sero positive pigs.
Pigs infected with pseudorabies field strains (mostly adult lately infected pigs) or vaccinated with gE+ vaccine, produce antibodies against pseudo rabies glycoprotein gE.
This test kit is designed to detect these antibodies against this gE glycoprotein by use of a blocking Enzyme Immuno Assay (ELISA). This test kit meets the requirements of the EC-program.

2. Intended use of the test kit

The principle of the test is based on the reaction of 2 monoclonal antibodies with 2 different antigenic determinants on the gE glycoprotein of Aujeszky's disease virus (ADV). Whereas a negative sample does not block the reaction, a sample containing antibodies to gE does block the reaction. However, pigs immunized with vaccines lacking gE expression do not block the reaction and thus are scored negative. In this way naturally, infected pigs can be discriminated from vaccinated pigs.

3. Principle of the test kit

A first monoclonal anti-gE antibody (the catching antibody) is used for coating the wells of a 96-well microtiter plate. Test sample and antigen, consisting of ADV infected cell cultures, are incubated in the microtiter test plate after 2 hours. A second monoclonal anti gE antibody, conjugated with horseradish peroxidase (HRPO) is added to the wells. This monoclonal antibody recognizes a different antigenic determinant on gE compared to the catching antibody.

- **Qualitative**
  The sample is added (diluted 1:1) to the wells of the coated plate.
- **Quantitative**
  The sample also can be titrated using a 3-step dilution, starting with a dilution 1:1(→ 1:3 → 1:9 → 1:27).

After incubation and washing, the substrate is added. If the test sample is negative, i.e. does not contain antibody to gE, HRPO and substrate will produce a colour reaction.
If the test sample is positive, the binding of the antigen to one or both monoclonal antibodies will be blocked and the colour reaction fails to appear.
A test sample is defined positive if the extinction is below 68% of the mean of that of the negative control which is included in this test kit.

4. Contents

- 5 x 12 x 8 Microtiter strips coated with the monoclonal catching antibody
- 5 x Strip holder
- 2 x 60 ml ELISA buffer (green cap)
- 1 x 60 ml HRPO conjugated anti-species antibodies (red cap)
- 1 x 0,5 ml Positive control (freeze dried) (purple cap)
- 1 x 1,0 ml Negative control (freeze dried) (silver cap)
- 1 x 60 ml Wash solution (200x concentrated) (black cap), **dilute in de-ionized water before use!**
- 1 x 60 ml Substrate A (white cap)
- 1 x 60 ml Substrate B (blue cap)
- 1 x 60 ml Stop solution (yellow cap)
- 5 x Plastic cover seal
- 1 x User's manual

Supplies needed (not included)

- Round-bottomed microtiter plate
- Validated precision pipettes
- Pipette tips and clean containers/tubes (EVL)
- ELISA plate reader

5. Handling and storage of specimens

The kit should be stored at 4°C.
An open packet should be used within 10 days.
Samples may be used fresh or may be kept frozen below -20°C before use.
After first use ready-to-use controls and/or reconstituted controls should be aliquoted immediately and stored at -20°C.
Avoid repeated freezing and thawing as this increases non-specific reactivity.

6. Wash protocol

In ELISA's, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better result.

Manual washing

1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µl wash solution.
3. This washing cycle (step 1 and 2) should be carried out at least 5 times.
4. Turn the plate upside down and empty the wells with a firm vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual wash solution in the wells.
6. Take care that none of the wells dry out before the next reagent is added.

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are aspirated completely and that the wash solution is correctly added, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 5 washing cycles.

7. Preparations

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 min. at room temperature (± 21°C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/ mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4-8°C immediately after use.

8. Test protocol qualitative

Before starting this test read "preparations"

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4°C. and use them within 10 days.

   Wash microtiter strip(s) with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 mega Ohm) water !
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Colostrum samples must be centrifuged for 15 minutes at 2000g to remove the lipid layer. Take the colostrum sample from under the lipid layer.

3. Reconstitute directly before use the **positive control** (purple cap) **in 0,5 ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
4. Reconstitute directly before use the **negative control** (silver cap) **in 1,0ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.

5. Make 3-step dilution of the **positive control** (purple cap) **in ELISA buffer** (green cap), **starting 1:1 → 1:3 → 1:9 → 1:27** in a round-bottomed microtiter plate (not supplied).
   Example: - Add 120µl ELISA buffer to **row 1A** and 120µl buffer to all other wells **1B, 1C, 1D**.
   - Add 120µl of the positive control to the well **1A.**
   - Mix well and transfer 60µl to the well **1B.**
   - Mix well and transfer 60µl to the well **1C.**
   - Mix well and transfer 60µl to the well **1D.**
   - Mix well and discard 60µl
6. Dilute the **negative control** (silver cap) **1:1 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 60µl ELISA buffer to **well 1E**, add 60µl of the negative control to the **well 1E** and mix well.
7. Dilute **each sample 1:1 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 60µl ELISA buffer to **well 1F**, add 60µl of the sample to the **well 1F** and mix well.
8. Take 2 wells as **substrate controls** add only **100µl ELISA buffer** (green cap) to these wells.

9. Transfer 100µl of all dilutions to the coated microtiter strips, including the substrate controls.
10. Seal and incubate for 75 minutes at 37°C. (Or overnight 12-18 hours at 4 °C).

11. Add **100µl HRPO conjugated anti-species antibodies** to all wells. (DON'T WASH)
12. Seal and incubate again for 60 min at 37°C.

13. Wash the plate according to the wash protocol [see sub 6].

14. Mix equal parts of substrate A (white cap) and substrate B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed**

15. Add **100µl substrate solution** to each well.
16. Incubate 10-20 min.in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21°C.). Make sure the negative control does not become too dark.

17. Add **50µl stop solution** to each well; mix well.
18. Read the absorbency values immediately (within 10 min!) at 450 nm by using an ELISA reader. **Use the substrate controls as blank.**

    N.B. Contaminated or lipemic sera can result in very high or low OD. Pigs of ≤ 6 months might have maternal antibodies.

    NB: if you pipet directly into the coated ELISA plate with only a small number of samples (<6), make sure the first dilution is done in round-bottom microtiter plate second step can be done directly in the coated Elisa plate.

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9. Test protocol quantitative

Before starting this test read "preparations"

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4°C. and use them within 10 days.

   Wash microtiter strip(s) with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 mega Ohm) water ! Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Colostrum samples must be centrifuged for 15 minutes at 2000g to remove the lipid layer. Take the colostrum sample from under the lipid layer.

3. Reconstitute directly before use the **positive control** (purple cap) **in 0,5 ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
4. Reconstitute directly before use the **negative control** (silver cap) **in 1,0ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.

5. Make 3-step dilution of the **positive control** (purple cap) **in ELISA buffer** (green cap), **starting 1:1 → 1:3 → 1:9 → 1:27** in a round-bottomed microtiter plate (not supplied).
   Example: - Add 120µl ELISA buffer to **row 1A** and 120µl buffer to all other wells **1B, 1C, 1D**.
   - Add 120µl of the positive control to the well **1A.**
   - Mix well and transfer 60µl to the well **1B.**
   - Mix well and transfer 60µl to the well **1C.**
   - Mix well and transfer 60µl to the well **1D.**
   - Mix well and discard 60µl.
6. Make 3-step dilution of the **negative control** (silver cap) **in ELISA buffer** (green cap), **starting 1:1 → 1:3 → 1:9 → 1:27** in a round-bottomed microtiter plate (not supplied).
   Example: - Add 120µl ELISA buffer to **row 1E** and 120µl buffer to all other wells **1F,1G,1H**
   - Add 120µl of the negative control to the well **1E**.
   - Mix well and transfer 60µl to the next well **1F**.
   - Mix well transfer 60µl to the next well **1G.**
   - Mix well and transfer 60µl to the well **1H.**
   - Mix well and discard 60µl.
7. Make 3-step dilution of **each sample in ELISA buffer** (green cap), **starting 1:1 → 1:3 → 1:9 → 1:27** in a round-bottomed microtiter plate (not supplied).
   Example: - Add 120µl ELISA buffer to the other **row 2A and 2E** (depending on the number of samples) and 120µl buffer to all other wells **2B, 2C, 2D / 2F, 2G, 2H**.
   - Add 120µl of the samples to the well **2A and/or 2E.**
   - Mix well and transfer 60µl to the well **2B and/or 2F**.
   - Mix well and transfer 60µl to the well **2C and/or 2G.**
   - Mix well and transfer 60µl to the well **2D and/or 2H.**
   - Mix well and discard 60µl.
8. Take 2 wells as **substrate controls** add only **100µl ELISA buffer** (green cap) to these wells.

9. Transfer 100µl of all dilutions to the coated microtiter strips, including the substrate controls.
10. Seal and incubate for 75 minutes at 37°C. (Or overnight 12-18 hours at 4 °C).

11. Add **100µl HRPO conjugated anti-species antibodies** to all wells. Mix well. (DON'T WASH)
12. Seal and incubate for 60 min at 37°C.

13. Wash the plate according to the wash protocol [see sub 6].
14. Mix equal parts of buffer A (white cap) and buffer B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.**

15. Add **100µl substrate solution** to each well.
16. Incubate 10-20 min.in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21°C.). Make sure the negative control does not become too dark.

17. Add **50µl stop solution** to each well; mix well.
18. Read the absorbency values immediately (within 10 min!) at 450 nm by using an ELISA reader. **Use the substrate controls as blank.**

    N.B. Contaminated or lipemic sera can result in very high or low OD. Pigs of ≤ 6 months might have maternal antibodies.

10. Precautions

- Handle all biological material as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal, results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

Validation of the test

Qualitative:

- The results are valid if the following criteria are met:
  - The mean value (MV) of the measured OD value for the Positive Control (PC), diluted 1:1, must be ≤0.500.
  - The MV of the measured OD value for the Negative Control (NC), diluted 1:1, must be ≥0.900.

  In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

  Calculation
  Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

  The ratio (S/N) of sample OD to mean OD of the negative control is calculated according to the following equation:

  $$S/N = \frac{OD_{sample}}{MV\ OD_{NC}}$$

Quantitative:
In order to confirm appropriate test conditions, the OD of the positive control, diluted 1:1, should be ≤0.500 OD units (450 nm) and give an endpoint titer of ≥3.
The negative control, diluted 1:1, should ≥0900 OD units (450 nm) and give an endpoint titer of ≤3.

11. Interpretation of the test results

This test can be used in 2 ways.

Qualitative: Positive – Negative

- A sample with the S/N ratio >0.68 is negative.
  - Specific antibodies to Aujeszky's disease virus could not be detected.
- A sample with the S/N ratio ≤ 0.68 is positive.
  - Specific antibodies to Aujeszky's disease virus were detected.

Quantitative: End point titre

- The ELISA titre can be calculated by constructing a curve and using cut-off line(dilution 1:1- 3-9 -27-81 -243 etc total 8 dilutions of 3 steps) OD on Y-as and titre on X-as.
  Elisa titres can be calculated using as cut-off the OD value of the 1:1 diluted negative control multiplied by the factor 0,68.

Note: Diseased animals that are positive in this test and are showing signs suggestive of Pseudorabies Virus are considered positive and must be suspected of shedding ADV in doubtful cases retest within 2-3 weeks with a fresh sample.

12. Symbols used with EVL ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructionsforuse	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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