Instructions for Use

Aujeszky's Disease Virus gE Ab ELISA

VET

REF EIA-2493

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

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A monoclonal antibody-mediated ELISA for the detection of Pseudo rabies Virus (Aujeszky's Disease Virus) gE antibodies in serum, plasma or colostrum samples

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 pseudorabies 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. 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 than the catching antibody.

- **Qualitative**

The sample is added (diluted 1:2) to the wells of the coated plate.

- **Quantitative**

The sample also can be titrated using a 2-step dilution, starting with a dilution undiluted 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 60% of the mean of that of the negative control which is included in this test kit.

4 CONTENTS

- 12 x 8 Microtiter strips coated with the monoclonal catching antibody
- 1 x strip holder
- 1 x 14 mL ELISA buffer (white bottle + green cap)
- 1 x 7.5 mL Inactivated antigene (freeze-dried) (green cap)
- 1 x 12 mL HRPO conjugate anti-species antibodies (**ready to use**) (black bottle + red cap)
- 1 x 0.5 mL Positive control serum (freeze-dried) (purple cap)
- 1 x 1.0 mL Negative control serum (freeze-dried) (silver cap)
- 1 x 20 mL Wash solution (200 x concentrated) (white bottle + black cap), **dilute in deionized water before use!**
- 1 x 8 mL Substrate Buffer A (white bottle + white cap)
- 1 x 8 mL Substrate Buffer B (black bottle + blue cap)
- 1 x 8 mL Stop Solution (white bottle + yellow cap)
- 1 x Plastic cover seal
- 1 x Instructions for Use

4.1 Supplies needed (not included)

- ELISA plate reader
- Pipette tips and clean containers/tubes
- Precision pipette 10-200µl
- Precision pipette 1-10µl
- Precision pipette 200-1000µl
- Round bottomed microtiter plate

5 VALIDATED PRECISION PIPETTES HANDLING AND STORAGE OF SPECIMENS

The kit should be stored at 4 °C.

An open packet should be used within 20 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 ELISAs, 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 results.

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 (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. Make sure that none of the wells dry out before the next reagent is dispensed.

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 dispensed, 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 minutes 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 °C - 8 °C immediately after use.

8 TEST PROTOCOL QUALITATIVE

1. Before starting this test read “PREPARATIONS”
2. 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 20 days.
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
3. Before testing make sure all reagents are at room temperature.
4. Wash the wells as pointed out in wash protocol. (**Dilute the washing fluid 1:200 in aquabidest before use**).
5. Colostrum samples must be centrifuged for 15 minutes at 2000*g* to remove the lipid layer.
   Take the colostrum sample from under the lipid layer.
6. Reconstitute directly before use the **positive control** (purple cap) in **0.5 mL** aqua bidest (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use, avoid freeze and thaw cycles.
7. Reconstitute directly before use the **negative control** (silver cap) in **1.0 mL** aqua bidest (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use, avoid freeze and thaw cycles.
8. Take out the amount of strips needed. Reconstitute this antigen vial with 8 mL ELISA buffer diluted 1:6 in demiwater. After complete reconstitution add **75 µL ADV antigen** to every well of the ELISA strips and incubate **75 minutes** at **37°C**.
9. 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 **120 µL ELISA buffer** (green cap) to **well 1A** and 120 µL buffer to all other wells **1B, 1C, 1D**.
   - Add 120 µL of the **positive control** to the well **1A.**
   - Mix well and transfer 60 µL to the well **1B.**
   - Mix well and transfer 60 µL to the well 1**C.**
   - Mix well and transfer 60 µL to the well **1D.**
   - Mix well and discard 60 µL.
10. Dilute the **negative control** (silver cap) **1:1 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
    Example: - Add 60 µL ELISA buffer (green cap) to **well 1E**, add 60 µL of the **negative control** to the **well 1E** and mix well.
11. Dilute **each sample 1:1 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
    Example: - Add 60 µL ELISA buffer (green cap) to **well 1F**, add 60 µL of the sample to the **well 1F** and mix well.
12. Take 2 wells as **substrate controls** add only **100 µL ELISA buffer** (green cap) to these wells.
13. Empty all the wells with ADV antigen and tap completely dry.
14. Transfer 100 µL of all dilutions to the **coated** microtiter strips, including the substrate controls.
15. Seal and incubate for **60 minutes** at **37 °C**. (Or overnight 12 - 18 hours at 4 °C).
16. Wash the strips 5x according to the wash protocol see sub 6.
17. Add **100 µL HRPO conjugated anti-species antibodies** to all wells.
18. Seal and incubate for **60 minutes** at **37 °C**.
19. Wash the strips 5x according to the wash protocol see sub 6.
20. 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.**
21. Add **100 µL substrate solution** to each well.
22. Incubate 10-15 minutes 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.
23. Add **50 µL stop solution** to each well; mix well.
24. Read the absorbency values immediately (within 10 minutes!) at 450 nm using 620 nm as reference on the ELISA reader. **Use the substrate controls as blank**.

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.

9 TEST PROTOCOL QUANTITATIVE

1. Before starting this test read “PREPARATIONS”
2. 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.
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
3. Before testing make sure all reagents are at room temperature.
4. Wash the wells as pointed out in wash protocol. (**Dilute the washing fluid 1:200 in aquabidest before use**)**.**
5. Colostrum samples must be centrifuged for 15 minutes at 2000*g* to remove the lipid layer.
   Take the colostrum sample from under the lipid layer.
6. Reconstitute directly before use the **positive control** (purple cap) in **0.5 mL** aqua bidest (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use, avoid freeze and thaw cycles.
7. Reconstitute directly before use the **negative control** (silver cap) in **1.0 mL** aqua bidest (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use, avoid freeze and thaw cycles.
8. Take out the amount of strips needed. Reconstitute this antigen vial with **8 mL ELISA buffer** diluted 1:6 in demiwater. After complete reconstitution add **75 µL ADV antigen** to every well of the ELISA strips and incubate **75 minutes** at **37°C**.
9. Make a **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 plate (not supplied).
   Example: - Add **120 µL** ELISA buffer (green cap) to **well 1A** and 120 µL buffer to all other wells **1B, 1C, 1D**.
   - Add 120 µL positive control to the well **1A**.
   - Mix well and transfer 60 µL to the well **1B**
   - Mix well and transfer 60 µL to the well **1C**
   - Mix well and transfer 60 µL to the well **1D**
   - Mix well and discard 60 µL.
10. Make a **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 plate (not supplied).
    Example: - Add **120 µL** ELISA buffer (green cap) to **well 1E** and 120 µL buffer to all other wells **1F, 1G, 1H**.
    - Add 120 µL negative control to the well **1E**.
    - Mix well and transfer 60 µL to the next well **1F**
    - Mix well and transfer 60 µL to the next well **1G**
    - Mix well and transfer 60 µL to the well **1H**
    - Mix well and discard 60 µL.
11. Make **3-step dilutions** of each **sample** in ELISA Buffer (green cap), starting **1:1 → 1:3 → 1:9 → 1:27** in a round bottomed plate (not supplied).
    Example: - Add **120 µL** ELISA buffer (green cap) to the **wells 2A and 2E** (depending on the number of samples)
    and 120 µL buffer to all other wells **2B, 2C, 2D / 2F, 2G, 2H**.
    - Add 120 µL of the samples to the well **2A and/or 2E**.
    - Mix well and transfer 60 µL to the well **2B and/or 2F**
    - Mix well and transfer 60 µL to the well **2C and/or 2G**
    - Mix well and transfer 60 µL to the well **2D and/or 2H**
    - Mix well and discard 60 µL.
12. Take 2 wells as substrate controls, add only 100 µL ELISA buffer (green cap) to these wells.

13. Empty all the wells with ADV antigen and tap completely dry.
14. Transfer 100 µL of all dilutions to the **coated** microtiter strips, including the substrate controls.
15. Seal and incubate for **60 minutes** at 37 °C. (Or overnight 12 - 18 hours at 4 °C.)
16. Wash the strips 5x according to the wash protocol [see sub 6].
17. Add 100 µL HRPO conjugated anti spezies antibodies to all wells. Mix well.
18. Seal and incubate for 60 minutes at 37 °C.
19. Wash the strips 5x according to the wash protocol [see sub 6].
20. 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**
21. Add 100 µL substrate solution to each well.
22. Incubate 10 - 15 minutes 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.
23. Add 50 µL stop solution to each well; mix well.
24. Read the absorbency values immediately (within 10 minutes!) at 450 nm using 620 nm as reference on the ELISA reader. Use the substrate controls as blank.

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

10 PRECAUTIONS

- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- Do not pipette by mouth.
- Do not use components past the expiry date and do not mix components from different serial lots.
  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.
- Handle all biological material as though capable of transmitting infectious diseases.
- 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.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.

11 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 1:2 must be ≤ 0.500.
  - The MV of the measured OD value for the Negative Control (NC) diluted 1:2 must be ≥ 0.800

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 1:2 should be ≤ 0.500 OD units (450 nm) and give an endpoint titer of ≥ 3.

The negative control diluted 1:2 should be ≥ 0.800 OD units (450 nm) and give an endpoint titer of ≤ 3.

12 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 a cut-off-line (dilution: 1:1 - 1:3 - 1:9 - 1:27 - 1:81 - 1:243, etc., total 8 dilutions of 3 steps) OD on Y-axis and Titre on X-axis.
  ELISA titres can be calculated using as cut-off the OD value of the undiluted negative control multiplied by the factor 0.68.

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use	Gebrauchsanweisung beachten	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device	*In-vitro*-Diagnostikum	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number	Katalognummer	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code	Chargenbezeichnung	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests	Ausreichend für <n> Prüfungen	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit	Temperaturgrenzwerte	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date	Verwendbar bis	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer	Hersteller	Fabbricante	Fabricante	Fabricant
	Distributor *	Vertriebspartner *	Distributore	Distribuidor	Distributeur
	Date of manufacture	Herstellungsdatum	Data di produzione	Fecha de fabricación	Date de production
	Biological risks	Biologische Risiken	Rischi biologici	Riesgos biológicos	Risques biologiques
	Caution	Achtung	Attenzione	Precaución	Attention
UDI	Unique device Identifier	eindeutige Produktidentifizierung			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Content	Content	Inhalt	Contenuto	Contenido	Contenu
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité