Instructions for Use

Feline Herpes Virus Ab ELISA

VET

REF EIA-2472

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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.

Introduced modifications / Durchgeführte Änderungen / Modifiche introdotte / Modificaciones introducidas /	
The following changes have been made in comparison to the previous version: Im Vergleich zur Vorgängerversion wurden folgende Änderungen vorgenommen: Rispetto alla versione precedente, sono state apportate le seguenti modifiche: Se han introducido los siguientes cambios en comparación con la versión anterior:	
8 TEST PROTOCOL QUALITATIVE:	Changed dilution for **positive control**: **1:100** → **1:300** → **1:900**→ **1:2700** (old: 1:50 → 1:150 → 1:450 → 1:1350);
9 TEST PROTOCOL QUANTITATIVE:	– From step 11 to step 16 changed pipetting volumes, – Changed **3-step dilution for controls and samples: 1:100** → **1:300** → **1:900**→ **1:2700** (old: 1:50 → 1:150 → 1:450 → 1:1350);
11 VALIDATION OF THE TEST:	updated
12 INTERPRETATION OF TEST RESULTS:	updated

Table of Contents

An ELISA to detect IgG antibodies against Feline Herpesvirus in serum or plasma samples of Feline species

1 INTRODUCTION

Demonstration of serum antibodies is the most commonly used method for the diagnosis of Feline Herpesvirus (FHV) infection or for monitoring the efficacy of vaccination. Feline Herpesvirus plays an important role in a complex disease of both wild and domestic cats ("sneezing disease"). The DNA virus replicates in respiratory, nasal, pharyngeal throat but also on conjunctival epithelial. As with other Herpesviruses, neuronal latency develops after primary infection. Stress and other factors might induce reinfection at this later neurological stage.

Important in diagnosis of Herpes infections:

- Clinical history
- Clinical signs
- Eye and mouth examination
- Laboratory findings

2 INTENDED USE OF THE TESTKIT

The FHV ELISA test is designed to detect antibodies against FHV proteins. FHV antigens (proteins/glycoproteins) are attached to the solid phase. After washing, the strips are incubated with the samples to be tested. The strips are washed after incubation to remove unbound materials. A HRPO labelled anti-species conjugate is added to detect bound cat antibodies to FHV proteins.

After incubation and rinsing the substrate is added and the optical density is measured at 450 nm.

3 PRINCIPLE OF THE TEST KIT

The test is based on the reaction of FHV proteins with polyclonal cat antibodies. To this end FHV antigens have been coated to a microtiter plate.

The diluted cat serum/plasma sample is added to the wells of the coated plate.

➢ Qualitative

The serum/plasma sample is added (diluted 1:150) to the wells of the coated plate.

➢ Quantitative

The sample also can be titrated using a 3-step dilution,
starting with a dilution 1:100→1:300→1:900→1:2700

After washing the bound cat antibodies are detected by a HRPO conjugated anti-species conjugate

The colour reaction in the wells is directly related to the concentration of FHV antibodies in serum/plasma samples.

4 CONTENTS

12 x 8 **Microtiter strips** coated with FHV proteins

1 x Strip holder

1 x 18 mL **ELISA Buffer** (white bottle + green cap)

1 x 12 mL **HRPO conjugate (IgG),** 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** (200x concentrated) (white bottle + black cap); Dilute in de-ionized 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
- Round bottomed microtiter plate
- Validated precision pipettes

5 HANDLING AND STORAGE OF SPECIMENS

After first use, ready-to-use controls and/or reconstituted controls should be aliquoted immediately and stored at -20 °C.

An opened packet should be used within 20 days.

Avoid repeated freezing and thawing as this increase non-specific reactivity.

Samples may be used fresh or may be kept frozen below -20 °C before use.

The kit should be stored at 2 °C - 8 °C.

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 washing 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 washing solution in the wells.
6. Take care 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 washing 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 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, store them at 4 °C and use them within 20 days.
   Use validated precision pipettes and use clean pipette tips before pipetting the buffer, control, samples, conjugate and substrate.
3. Before testing make sure all reagents are at room temperature.
4. The washing solutions provided must be diluted **1:200** in aqua bidest before use.
   Wash the wells as pointed out in wash protocol.
5. 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.
6. 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.
7. Dilute the **positive control** (purple cap) **starting 1:100 → 1:300 → 1:900→ 1:2700** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - A pre-dilution is needed:
   - Add 90 µL ELISA buffer to **well 1A**, add 10 µL of the positive control to the **well 1A** and mix well.
   - Add 180 µL ELISA buffer to **well 2A**,
   - Add 120 µL ELISA buffer to **wells 2B, 2C, and 2D.**
   - Add 20 µL of pre-dilution from **well 1A** in the well **2A**.
   - Mix well and transfer 60 µL to the well **2B**.
   - Mix well and transfer 60 µL to the well **2C.**
   - Mix well and transfer 60 µL to the well **2D**.
   - Mix well and discard 60 µL.
8. Dilute the **negative control** (silver cap) **1:150** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - A pre-dilution is needed:
   - Add 90 µL ELISA buffer to **well 1E**, add 10 µL of the negative control to **well 1E** and mix well.
   - Add 140 µL ELISA buffer to **well 2E**, add 10 µL of pre-dilution from well 1E to **well 2E** and mix well.
9. Dilute each **sample 1:150** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - A pre-dilution is needed:
   - Add 90 µL ELISA buffer to **well 1F**, add 10 µL of the sample to **well 1F** and mix well.
   - Add 140 µL ELISA buffer to **well 2F**, add 10 µL of pre-dilution from well 1F in **well 2F** and mix well.
10. Take 2 wells as substrate controls, add only 120 µL ELISA buffer (green cap) to these wells.

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11. Transfer 100 µL of all dilutions from **column 2** to the virus **coated** microtiter strips, including the substrate controls.
12. Seal and incubate for 60 minutes at 37 °C.
13. Wash the strips incubated with antigen 5x according to the wash protocol. see chapter 6.
14. Add 100 µL HRPO conjugated anti-species antibodies (red cap) to all wells.
15. Seal and incubate for 60 minutes at 37 °C.
16. Wash the strips 5x according to the wash protocol. see chapter 6..
17. 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.**
18. Add 100 µL substrate solution to each well.
19. 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.
20. Add 50 µL Stop Solution to each well; mix well.
21. 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 20 days.
   Use validated precision pipettes and use clean pipette tips before pipetting the buffer, control, samples, conjugate and substrate.
3. Before testing make sure all reagents are at room temperature.
4. The washing solutions provided must be diluted **1:200** in aqua bidest before use.
   Wash the wells as pointed out in wash protocol.
5. 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.
6. 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.
7. Make a pre-dilution of the **positive control** (purple cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 90 µL ELISA buffer to **well 1A** and add 10 µL of the positive control to the well **1A.**
8. Make a pre-dilution of the **negative control** (silver cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 90 µL ELISA buffer to **well 1B** and add 10 µL of the negative control to the well **1B.**
9. Make a pre-dilution of **each sample** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 90 µL ELISA buffer to **well 1C** and add 10 µL of the sample to the well **1C.**
10. Take 2 wells as substrate controls, add only 120 µL ELISA buffer (green cap) to these well.

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11. Add for dilution of the **positive control** 135 µL ELISA buffer to **well 1A.**
    And add 100 µL to **1B, 1C, 1D** of the **coated** microtiter strip.
12. Add for dilution of the **negative control** 135 µL ELISA buffer to **well 1E.**
    And add 100 µL to **1F, 1G, 1H** of the **coated** microtiter strip.
13. Add for dilution of the **samples** 135 µL ELISA buffer to the other **wells 2A and 2E.**
    And add 100 µL to **2B, 2C, 2D and 2F, 2G, 2H** (depending on the number of samples) of the **coated** microtiter strip.
14. Make a 3-step dilution of the **positive control** in the coated microtiter strip,
    starting 1:100 → 1:300 → 1:900 → 1:2700.
    Example: **-** Add 15 µL positive control from step 7 to the well **1A** of the microtiter strip.
    - Mix well and transfer 50 µL to the well **1B**
    - Mix well and transfer 50 µL to the well **1C**
    - Mix well and transfer 50 µL to the well **1D**
    - Mix well and discard 50 µL.
15. Make a 3-step dilution of the **negative control** in the coated microtiter strip,
    starting 1:100 → 1:300 → 1:900 → 1:2700.
    Example: **-** Add 15 µL negative control from step 8 to the well **1E** of the microtiter strip.
    - Mix well and transfer 50 µL to the next well **1F**
    - Mix well and transfer 50 µL to the next well **1G**
    - Mix well and transfer 50 µL to the well **1H**
    - Mix well and discard 50 µL.
16. Make a 3-step dilution of **each sample** in the coated microtiter strip,
    starting 1:100 → 1:300 → 1:900 → 1:2700.
    Example: **-** Add 15 µL of each sample from step 9 to the well **2A and/or 2E** of the microtiter strip.
    - Mix well and transfer 50 µL to the well **2B and/or 2F**
    - Mix well and transfer 50 µL to the well **2C and/or 2G**
    - Mix well and transfer 50 µL to the well **2D and/or 2H**
    - Mix well and discard 50 µL.
17. Add 100 µL of the substrate control from step 10 to the last 2 wells of the coated microtiter strip.
18. Seal and incubate for 60 minutes at 37 °C.

19. Wash the strips 5x according to the wash protocol. see chapter 6.
20. Add 100 µL HRPO conjugated anti-species antibodies (red cap) to all wells.
21. Seal and incubate 60 minutes at 37 °C.
22. Wash the strips according 5x to the wash protocol. see chapter 6..
23. 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.
24. Add 100 µL substrate solution to each well.
25. 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.
26. Add 50 µL Stop Solution to each well; mix well.
27. 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.

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 1:100, must be ≥ 0.850.
- The mean value (MV) of the measured OD value for the negative control (NC), diluted 1:150, must be ≤ 0.350.

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/P) of sample OD to mean OD of the positive control is calculated according to the following equation:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Quantitative:

In order to confirm appropriate test conditions, the OD of

- the positive control, diluted 1:100, should be ≥ 0.850 OD units (450 nm) and give an endpoint titer of ≥ 0.100.
- the negative control, diluted 1:100, should be ≤ 0.350 OD units (450 nm) and give an endpoint titer of ≤ 100.

12 INTERPRETATION OF TEST RESULTS

This test can be used in 2 ways.

Qualitative: Positive - Negative

- A sample with the S/P ratio **< 0.23** is negative.
  - Specific antibodies to Feline Herpes virus could not be detected
- A sample with the S/P ratio **≥ 0.23** is positive.
  - Specific antibodies to Feline Herpes virus were detected

Quantitative: End point titre

- The ELISA titre can be calculated by constructing a curve and using a cut-off line (dilution 1:100 → 1:300 → 1:900 → 1:2700, 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 2.5 times OD value of negative control at 1:100.

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
	Batch code *	Chargencode *	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 *	Temperaturbegrenzung *	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
	Caution *	Achtung *			
VET	For veterinary use only				
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Contenu
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité