Instructions for Use

Feline Calici Virus Ab ELISA

EIA-2473

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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.

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

1 INTRODUCTION

For Veterinary use only!

Demonstration of serum antibodies is the most commonly used method for the diagnosis of Feline Calici Virus (FCV) infection or for monitoring the efficacy of vaccination. Feline Calici plays an important role in a complex disease of both wild and domestic cats ("sneezing disease"), nasal discharge, conjunctivitis and ulceration of the tong.

Signs may last from days to weeks and vary in severity. In young kittens pneumoniae is often seen.

Important in the diagnosis of Feline Calici infection:

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

2 INTENDED USE OF THE TEST KIT

The FCV ELISA test is designed to detect antibodies against Feline Calici Virus proteins. To this end Feline Calici proteins are attached to the solid phase. The strips are washed after incubation to remove unbound materials. The samples to be tested are diluted and added to the wells and incubated. After incubation the strips are washed, a HRPO labeled anti-species conjugate is added to detect bound cat antibodies to FCV. 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 FCV proteins with polyclonal cat antibodies.

To this end FCV proteins have been attached to the solid phase.

- **Qualitative**
  The 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 is directly related to the concentration of FCV antibodies in serum/plasma samples.

4 CONTENTS

- 12 x 8 **Microtiter strips** coated with purified FCV glycoproteins
- 1 x Strip holder
- 1 x 18 mL **ELISA Buffer** (green cap)
- 1 x 12 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 20 mL **Wash solution** (200x concentrated), **dilute in deionized water before use!**
- 1 x 8 mL **Substrate buffer A** (white cap)
- 1 x 8 mL **Substrate buffer B** (blue cap)
- 1 x 8 mL **Stop Solution** (yellow cap)
- 1 x Plastic cover seal
- 1 x Instructions for use

4.1 Supplies needed (not included)

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

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 open packet should be used within 20 days.

Avoid repeated freezing and thawing of all the products as this increases 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 an ELISA, 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 (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 downward 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 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 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 2 °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 2 °C - 8 °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 aqua bidest. before use).
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**,
   - and 120 µL ELISA buffer to **2B, 2C, and 2D.**
   - Add 20 µL of pre-dilution from well 1A in the well **2A** and mix well.
   - Mix well 2A and transfer 60 µL to the well **2B**.
   - Mix well 2B and transfer 60 µL to the well **2C**.
   - Mix well 2C and transfer 60 µL to the well **2D**.
   - Mix well 2D 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 (green cap) to **well 1E,** add 10 µL of the negative control to the **well 1E** and mix well
   - Add 140 µL ELISA buffer (green cap) to **well 2E,** add 10 µL of the pre-dilution of **well 1E** to the **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 the **well 1F** and mix well.
   - Add 140 µL ELISA buffer to **well 2F**, add 10 µL of the pre-dilution of well 1F to **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 srips 5x according to the wash protocol see chapter 6.
14. Add 100 µL HRPO conjugated anti-species antibodies to all wells.
15. Seal and incubate 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 pre-dilution step 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 2 °C - 8 °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. 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 negative control to the well **1C.**
10. Take 2 wells as substrate controls, add only 120 µL ELISA buffer (green cap) to these wells.

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11. Add for dilution of the **positive control** 135 µL ELISA buffer to **well 1A**,
    and 100 µL ELISA buffer 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 100 µL ELISA buffer 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 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 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 of step 10 to the last 2 wells of the 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 to all wells.
21. Seal and incubate 60 minutes at 37 °C.
22. Wash the strips according 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 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 MV of the measured OD value for the Negative Control (NC), diluted 1:100, 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 **titre of ≥ 100**.

The negative control, diluted 1:150, should be **≤ 0.350** OD units (450 nm) and give an endpoint **titre 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 Calicivirus could not be detected.
- A sample with the S/P ratio **≥ 0.23** is *positive*
  Specific antibodies to Calicivirus were detected.

Quantitative: End point titre

The ELISA titre can be calculated by constructing a curve and using 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 1:150.

The purchaser assumes the entire risk as to the performance of these products.
DRG shall not be liable for indirect, special or consequential damage of any kind resulting from use of these products.

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
REF	Catalogue number *	Artikelnummer *	No. di Cat.	No de catálogo	Référence
LOT	Batch code *	Chargencode *	Lotto no	Número de lote	No. de lot
Σ	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 *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
VET	For veterinary use only				
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Conditionnement
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité