FIV p24/p17 (Feline Immunodeficiency Virus) Ab ELISA

Enzyme Immunoassay for the detection antibodies against Feline Immunodeficiency Virus (FIV) p24 and p17 antigen (expression protein) in serum and plasma samples

REF DE2463

96 wells
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1 INTRODUCTION
FIV p17/p24 are both core proteins of FIV. Infected cats produce antibodies against these FIV antigens, which can be detected in an ELISA using an anti-species conjugate.

2 INTENDED USE OF THE TEST KIT
The FIV p17/p24 ELISA is designed to detect antibodies against these proteins. To this end recombinant p17/p24 proteins are attached to the solid phase. After washing the plates are incubated with the cat sera to be tested. The plates are washed after incubation to remove unbound materials. An anti-species conjugate is added to detect bound cat antibodies to FIV p17/p24. 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 FIV proteins with cat antibodies. To this end, p17/p24 expression proteins have been coated to a 96 well microtiter strip plate.

- **Qualitative**
The cat serum sample is added (diluted 1:100) to the wells of the coated plate.

- **Quantitative**
The cat serum 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 an anti-species conjugate. Bound anti-species conjugate is made visible by adding substrate/chromogen mix. The intensity of the color reaction in the wells is directly correlated to the concentration of anti-FIV p17/p24 antibodies in the serum sample.

4 CONTENTS
- SORB MT 12 x 8 Microtiter strips
- 1 x Strip holder
- BUF 1 x 18 mL ELISA buffer (green cap)
- ENZ CONJ 1 x 12 mL HRPO conjugated anti-species antibodies (red cap)
- CONTROL 1 1 x 0.5 mL Positive control (freeze dried) (purple cap)
- CONTROL 2 1 x 1.0 mL Negative control (freeze dried) (silver cap)
- WASH SOLN 200x 1 x 20 mL Wash-solution (200x concentrated) (black cap), diluted in de-ionized water before use!
- SUB A 1 x 8 mL Substrate A (white cap)
- SUB B 1 x 8 mL Substrate B (blue cap)
- STOP SOLN 1 x 8 mL Stop-solution (yellow cap)
- 1 x Plastic cover seal

4.1 Supplies needed (not included)
- Round bottomed microtiter plate
- Precision pipette 0.1-5 µL
- Precision pipette 10-200 µL
- Precision pipette 200-1000 µL
- Pipette tips and clean containers/tubes
- 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. Positive and negative controls may be stored after reconstitution in aliquots at -20 °C and used until the expiry date. 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 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 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 °C - 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) 5x with washing solution, according to washing protocol.

   **The washing solutions provided must be diluted 200x in aqua bidest. (5 MΩ water)**

   Use the precision pipette 0.1-5 µL, 10-200 µL & 200-1000 µL and use a clean pipette tip before pipetting the buffer, control, samples, conjugate and substrate.

2. 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.

3. 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.

4. Dilute the positive control (purple cap) 1:100 in ELISA buffer (green cap) in a round bottomed plate (not supplied). Make sure to make at least 125 µL of every dilution to be able to transfer 100 µL to the coated plate and minimize pipetting errors.

5. Dilute the negative control (silver cap) 1:100 in ELISA buffer (green cap) in a round bottomed plate (not supplied). Make sure to make at least 125 µL of every dilution to be able to transfer 100 µL to the coated plate and minimize pipetting errors.

6. Dilute the sample 1:100 in ELISA buffer (green cap) in a round bottomed plate (not supplied). Make sure to make at least 125 µL of every dilution to be able to transfer 100 µL to the coated plate and minimize pipetting errors.

7. Take 2 wells as substrate controls, add only 100 µL ELISA buffer (green cap) to these well.

8. Transfer 100 µL of all dilutions to the coated microtiter strips.

9. Seal and incubate for 60 min at 37 °C.

10. Wash the plate 5 times according to the wash protocol see sub 6.

11. Dispense 100 µL conjugated anti-species antibody to all wells.

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. Dispense 100 µL substrate solution to each well.

16. Incubate 10-15 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 50 µL stop solution to each well; mix well.

18. Read the absorbency values immediately (within 10 min) at 450 nm using 620 nm as reference on the ELISA reader. Use the substrate controls as blank.
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) 5x with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aqua bidest. (5 MΩ water)!

   Use the precision pipette 0.1–5 µL, 10–200 µL & 200–1000 µL and use a clean pipette tip before pipetting the buffer, control, samples, conjugate and substrate.

2. 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.

3. 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.

4. Make a 3-step dilution of the positive control (purple cap) in ELISA buffer (green cap) starting with 1:100 → 1:300 → 1:900 → 1:2700 in a round-bottomed microtiter plate (not supplied).

   Make sure to make at least 125 µL of every dilution to be able to transfer 100 µL to the coated plate and minimize pipetting errors.

5. Make a 3-step dilution of the negative control (silver cap) in ELISA buffer (green cap) starting with 1:100 → 1:300 → 1:900 → 1:2700 in a round-bottomed microtiter plate (not supplied).

   Make sure to make at least 125 µL of every dilution to be able to transfer 100 µL to the coated plate and minimize pipetting errors.

6. Make 3-step dilutions of each sample in ELISA buffer (green cap) starting with 1:100 → 1:300 → 1:900 → 1:2700 in a round-bottomed microtiter plate (not supplied).

   Make sure to make at least 125 µL of every dilution to be able to transfer 100 µL to the coated plate and minimize pipetting errors.

7. Take 2 wells as substrate controls add only 100 µL ELISA buffer (green cap) to these well.

8. Transfer 100 µL of all dilutions to the virus-coated microtiter strips.

9. Seal and incubate for 60 min at 37 °C.

10. Wash the plate 5 times according to the wash protocol see sub 6.

11. Dispense 100 µL conjugated anti-species antibody to all wells.

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. Dispense 100 µL substrate solution to each well.

16. Incubate 10-15 min in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21 °C). Make sure the negative does not become too dark.

17. Add 50 µL stop solution to each well; mix well.

18. Read the absorbency values immediately (within 10 min!) at 450 nm using 620 nm as reference on the ELISA reader. Use the substrate controls as blank.
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.

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) must be \( \geq 0.850 \)
  - The MV of the measured OD value for the Negative Control (NC) must be \( \leq 0.400 \)

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:

\[
S/P = \frac{\text{OD}_{\text{sample}} - \text{MV OD}_{\text{NC}}}{\text{MV OD}_{\text{PC}} - \text{MV OD}_{\text{NC}}}
\]

**Quantitative:**

- In order to confirm appropriate test conditions the OD of the positive control should be \( \geq 0.850 \) OD units (450 nm) and give an endpoint titer of \( \geq 300 \).
- The negative control should be \( \leq 0.400 \) OD units (450 nm) and give an endpoint titer of \( \leq 100 \).

12 INTERPRETATION OF THE TEST RESULTS

This test can be used in 2 ways.

**Qualitative:** Positive – Negative

- A sample with the S/P ratio < 0.4 is negative
  - Specific antibodies to FIV could not be detected.
- A sample with the S/P ratio \( \geq 0.4 \) is positive
  - Specific antibodies to FIV 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 \( \rightarrow 1:300 \rightarrow 1:900 \rightarrow 1:2700 \rightarrow 8100 \rightarrow 24300 \), 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.

The entire risk as to the performance of these products is assumed by the purchaser. Demeditec shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products in case of problems or questions contact Demeditec.
## SYMBOLS USED WITH DEMEDITEC ASSAYS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>English</th>
<th>Deutsch</th>
<th>Français</th>
<th>Español</th>
<th>Italiano</th>
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<td>European Conformity</td>
<td>CE-Konformitätskennzeichnung</td>
<td>Conforme aux normes européennes</td>
<td>Conformidad europea</td>
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<td>Gebrauchsanweisung beachten</td>
<td>Consulter les instructions d'utilisation</td>
<td>Consulte las Instrucciones</td>
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<td>In-vitro-Diagnostikum</td>
<td>Usage Diagnostic in vitro</td>
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- **European Conformity**: CE-Konformitätskennzeichnung
- **Consult instructions for use**: Gebrauchsanweisung beachten
- **In vitro diagnostic device**: In-vitro-Diagnostikum
- **For research use only**: Nur für Forschungszwecke
- **Catalogue number**: Katalog-Nr.
- **Lot. No. / Batch code**: Chargen-Nr.
- **Contains sufficient for <n> tests/**: Ausreichend für "n" Tests
- **Note warnings and precautions**: Warnhinweise und Vorsichtsmaßnahmen beachten
- **Storage Temperature**: Lagerungstemperatur
- **Expiration Date**: Mindesthaltbarkeitsdatum
- **Legal Manufacturer**: Hersteller
- **Distributed by**: Distributor