Instructions for Use

Feline Immunodeficiency Virus p24/p17 Ab ELISA

VET

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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 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.

Table of Contents

1 INTRODUCTION 2
2 INTENDED USE OF THE TEST KIT 2
3 PRINCIPLE OF THE TEST KIT 2
4 CONTENTS 2
5 HANDLING AND STORAGE OF SPECIMENS 3
6 WASH PROTOCOL 3
7 PREPARATIONS 3
8 TEST PROTOCOL QUALITATIVE 3
9 TEST PROTOCOL QUANTITATIVE 4
10 PRECAUTIONS 5
11 VALIDATION OF THE TEST 5
12 INTERPRETATION OF THE TEST RESULTS 6

SYMBOLS USED 7

An ELISA test to detect antibodies against Feline Immunodeficiency Virus (FIV) antigen in serum and plasma of cats.

1 INTRODUCTION

FIV is a lentivirus that was discovered in 1986 by Dr. Niels Pedersen at the University of California. The virus has a world-wide distribution with a prevalence of around 5% in healthy cats. FIV is transmitting mainly by biting. It can also be transmitted from mother to kitten during the prenatal period. The virus establishes a persistent infection from which cats usually recover. There follows an asymptomatic phase lasting several years in which the cat is clinically healthy. However, over time the immune function in the cat deteriorates and opportunistic infections (especially of the respiratory and gastrointestinal tracts, lymphomas or neurological disorders) arise.
Almost all cats infected with FIV have antibodies to viral structural proteins, particularly the envelope proteins (SU/TM) and the core proteins (p24/p17). Antibodies are first detected in serum 3-6 weeks after infection. Occasionally cats show an antibody response against a single envelope or core protein.
At the moment all attempts to develop a vaccine (until now, without success) are based on envelope proteins, in this way the detection of p24 antibodies is the only possibility to distinguish vaccinated cats from non-vaccinated cats in the future. Recent articles written indicate that the p24 response is significantly higher in clinical (reasonable) healthy cats (but FIV infected) which could mean that it can be monitored as illness progression protein.

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 samples 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 at450nm.

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 plate.

- **Qualitative**

The cat serum/plasma sample is added (diluted 1:100) to the wells of the coated plate.

- **Quantitative**

The cat serum/plasma 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 or plasma sample.

4 CONTENTS

- 12x 8 Microtiter strips coated with FIV proteins.
- 1x Strip holder
- 1x 18 mL ELISA buffer ((white bottle + green cap)
- 1x 12 mL HRPO conjugate (IgG) anti-species antibodies **(ready to use)** black bottle + red cap)
- 1x 0.5 mL Positive control serum **(freeze dried)** (purple cap)
- 1x 1.0 mL Negative control serum **(freeze dried)** (silver cap)
- 1x 20 mL Wash-solution (200x concentrated) (white bottle + black cap), **dilute in de-ionized water before use)**
- 1x 8 mL Substrate buffer A (white bottle + white cap)
- 1x 8 mL Substrate buffer B (black bottle + blue cap)
- 1x 8 mL Stop-solution (white bottle + yellow cap)
- 1x Plastic cover seal
- 1x Instructions for Use

4.1 Supplies needed (not included)

- ELISA plate reader
- Pipette tips and clean containers/tubes (EVL)
- Precision pipette 10-200µl (EVL)
- Precision pipette 1-10µl (EVL)
- Precision pipette 200-1000µl (EVL)
- 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 store at -20 °C.

An open packet should be used within 20 days.

Avoid repeated freezing and thawing 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 4°C.

6 WASH PROTOCOL

In 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 washing solution.
3. This washing cycle (1 and 2) should be carried out at least 5 times.
4. Turn the plate upside down 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. Make sure that none of the wells dries 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 the reagents back at 4 °C - 8 °C immediately after use.

8 TEST PROTOCOL <u>QUALITATIVE</u>

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. <u>Reconstitute</u> directly before use the **positive control** (purple cap) in **0,5 mL** aquabidest. (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use, avoid freeze and thaw cycles.

6. <u>Reconstitute</u> directly before use the **negative control** (silver cap) in **1,0 mL** aquabidest. (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use, avoid freeze and thaw cycles.

7. <u>Dilute</u> 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: - apre-dilution is needed:
   - Add 90 µL ELISA buffer (green cap) to **well 1A**, add 10 µL of the positive control to the **well 1A** and mix well.
   - Add 180 µL ELISA buffer (green cap) to **row 2A**
   - Add 120 µL ELISA buffer (green cap) to **2B, 2C, 2D**
   - Add 20 µL of pre-dilution **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. <u>Dilute</u> the **negative control** (silver cap) **1:100** 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 **row 1E**, add 10 µL of the negative control to the **well 1E** and mix well.
   - Add 135 µL ELISA buffer (green cap) to **row 2E**, add 15 µL of pre-dilution from **well 1E** in the well **2E** and mix well.

9. Dilute the **sample 1:100** 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 **row 1F**, add 10 µL of the sample to the **well 1F** and mix well.
   - Add 135 µL ELISA buffer (green cap) to **row 2F**, add 15 µL of pre-dilution from **well 1F** in the well **2F** and mix well
10. Take 2 wells as substrate controls, add only 120 µL ELISA buffer (green cap) to these wells.
11. Transfer 100 µL of all dilutions **of column 2** to the **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 sub 7.
14. Add 100 µL HRPO conjugated anti-species (red cap) antibodies to all wells.
15. Seal and incubate for 60 minutes at 37 °C.
16. Wash the strips 5x according to the wash protocol see sub 7.
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 10minutes!) 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 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** aquabidest. (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 **well 1A** and mix well.
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 (green cap) to **well 1B** and add 10 µL of the negative control to **well 1B** and mix well.
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 (green cap) to **well 1C** and add 10 µL of the sample to **well 1C** and mix well.
10. Take 2 wells as substrate controls, add only 120 µL ELISA buffer (green cap) to these well.
11. Add for dilution of the **positive control** 135 µL ELISA buffer (green cap) to **well 1A,** and 100 µL ELISA buffer (green cap) to the **wells 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 (green cap) to the **wells 1F, 1G, 1H** of the **coated** microtiter strip.
13. Add for dilution of **the samples** 135 µL ELISA buffer (green cap) to **well 2A and 2E** (depending on the number of samples), and 100 µL ELISA buffer (green cap) to the **wells 2B, 2C, 2D** and **wells 2F, 2G, 2H** 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 **well 1A** of the coated microtiter strip.
    - Mix well and transfer 50 µL to the well **1B**
    - Mix well and transfer 50 µL to the well 1**C**
    - 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 **well 1E** of the coated microtiter strip.
    - Mix well and transfer 50 µL to the well **1F**
    - Mix well and transfer 50 µL to the well 1**G**
    - 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 well **2A and/or 2E** of the coated 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 sub 7].
20. Add 100 µL HRPO conjugated anti-species antibodies (red cap) to all wells.
21. Seal and incubate for 60 minutes at 37 °C.
22. Wash the strips according to the wash protocol [see sub 7].
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 10minutes!) 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) must be ≤ 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:

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

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 ≥ 300.

The negative control diluted 1:100, should be ≤ 0.400 OD units (450 nm) and give an endpoint titre of ≤ 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 < 0.23 is negative.
  - Specific antibodies to Feline Immunodeficiency virus could not be detected.
- A sample with the S/P ratio ≥ 0.23 is positive.
  - Specific antibodies to Feline Immunodeficiency 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 →1:8100→ 1:24300 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. DRG 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 DRG.

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français	Português
	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes	Conformidade Europeia
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation	Consultar as instruções de uso
	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro	Dispositivo médico para diagnóstico in vitro
	Catalogue number *	Katalognummer *	No. di Cat.	No de catálogo	Référence	Número de catálogo
	Batch code *	Chargen-bezeichnung *	Lotto no	Número de lote	No. de lot	Código do lote
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per" n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests	Suficiente para <n> determinações
	Temperature limit *	Temperaturgrenzwerte *	Temperatura di conservazione	Temperatura de conservacion	Temperature de conservation	Limites de temperatura
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation	Prazo de validade
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant	Fabricante
	Distributor *	Vertriebspartner *	Distributore	Distribuidor	Distributeur	Distribuidor
	Date of manufacture *	Herstellungsdatum *	Data di produzione	Fecha de fabricación	Date de production	Data de fabricação
	Biological risks *	Biologische Risiken *	Rischi biologici	Riesgos biológicos	Risques biologiques	Riscos biológicos
	Caution *	Achtung *	Attenzione	Precaución	Attention	Cuidado
	Unique device Identifier *	eindeutige Produktidentifizierung *	Identificativo unico del dispositivo*	Identificación exclusiva del dispositivo *	Identifiant de dispositif unique*	Identificador único do dispositivo *
	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	Conditionnement	Conteúdo
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité	Volume / Quantidade