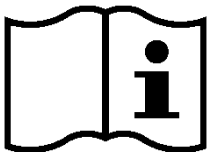

Useŕs Manual

Feline Immunodeficiency Virus p24 Antigen ELISA

An ELISA test to detect Feline Immunodeficiency Virus (FIV) p24 antigen in tissue culture samples

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Gebruik alleen de juiste versie van het protocol dat meegestuurd wordt met de kit.
Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung.
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.

Inhoud / Table of Contents / Inhaltsverzeichnis / Tabella die Contenuti / Tabla de Contenidos

1. Introduction

Vaccines for use in animals consist of inactivated antigen suspensions of in-vitro cultured virus. FIV in cats has been used as a model for HIV in humans. For this reason FIV vaccines have become of interest. To standardise antigen preparations, measurement of p24 is used. ELISA's are rapid and reproducible and the use of standardised test kits enables the user to determine the p24 contents of inactivated antigen preparations from lot to lot, easily and reliably.

2. Intended use of the test kit

This diagnostic test is intended for the detection of p24 nucleoprotein in tissue culture samples. This standardized ELISA is based on affinity purified polyclonal antibodies directed to p24.

3. Principle of the test kit

The test is based on the reaction of FIV p24 proteins with affinity purified HRPO labelled polyclonal antibodies. To this end, FIV p24 proteins have been attached to the solid phase. The tissue culture samples (diluted/ undiluted) are added to the wells of a pre-incubation plate. Immediately the same volume of HRPO labelled conjugate is added. After pre-incubation the antigen/conjugate mixture is transferred to the (washed) coated microtiter plate.
After washing a substrate/chromagen mix is added to detect the bound conjugate.
The intensity of the color reaction in the wells is inversely correlated to the concentration of FIV p24 in the sample.

4. Contents

- 12 x 8 Microtiter strips
- 1 x Strip holder
- 1 x 96 well non-coated round-bottomed microtiter plate
- 3 x 6 ml ELISA buffer (green cap)
- 2 x 6 ml HRPO labelled anti-FIV p24 antibodies (red cap)
- 1 x 1 ml Standard 1 (50 µg/ml).
- 1 x 60 ml Wash-solution (200x concentrated) (black cap), **dilute in de-ionized water before use!**
- 1 x 8 ml Substrate A (white cap)
- 1 x 8 ml Substrate B (blue cap)
- 1 x 8 ml Stop-solution (yellow cap)
- 1 x Plastic cover seal
- 1 x User's manual

Supplies needed (not included)

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

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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.
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 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 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 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. Take care that none of the wells dry out before the next reagent is added.

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 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 4-8°C immediately after use.

8. Test protocol

Before starting this test read "preparations"

1. Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Dilute the **standard** (yellow cap) **starting undiluted → 1:2 → 1:4 → 1:8 → 1:16 → 1:32 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 80µl ELISA buffer to **row 1B-1F**
   - Add 160µl of the positive control to the well **1A**
   - Add 80µl of **well 1A** in the well **1B**
   - Mix well and transfer 80µl to the well **1C**
   - Mix well and transfer 80µl to the well **1D**
   - Mix well and transfer 80µl to the well **1E**
   - Mix well and transfer 80µl to the well **1F**
   - Mix well and discard 80µl.
3. Dilute the **supernatant** of each tissue-culture sample (without Azide or HRPO inhibitors) **undiluted** and **diluted 1:10 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 80µl of the supernatant to row **2A**
   - Add 72µl ELISA buffer to **well 2B**, add 8µl of the sample to the **well 2B** and mix well.
4. Take 2 wells as **substrate controls** add only **80µl ELISA buffer** (green cap) to these wells.
5. Add immediately **75µl of HRPO labelled anti-FIV p24 antibodies** to all the wells.
6. Seal and incubate for 60 min at 37°C.

7. 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 aquabidest (5 mega Ohm) water!

8. Transfer 100µl of all dilutions to the FIV coated microtiter strips, including the substrate controls.
9. Seal and incubate for 60 min at 37°C.

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

11. 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.**

12. Add **100µl substrate solution** to each well.
13. Incubate 10-13 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.

14. Add **50µl stop solution** to each well; mix well.

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

9. 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.

10. Validation of the test

To standardize the FIV p24 ELISA standards 1 to 10 have to be included in each test. The FIV standard should give an extinction between 0.050 and 1.600 OD, measured at 450 nm using 620 nm as reference.

11. Interpretation of the test results

Plot a curve using the values obtained with the standards on log/log paper (OD values on Y-axis and µg/ml on X-axis). The concentration of the samples can be inferred from this standard curve (pay attention to the dilution factors).

12. Symbols used with EVL ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

The entire risk as to the performance of these products is assumed by the purchaser. EVL 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 EVL.

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