

ViraCHEK® FIV

Feline Immunodeficiency Virus Antibody Test Kit ELISA Test Kit For The Detection of Antibodies to Feline Immunodeficiency Virus

For Veterinary Use Only

32-94 reactions

I. INTRODUCTION

Feline Immunodeficiency Virus (FIV) is a lentivirus specifically pathogenic to cats. The Virus was originally described as a feline thymolymphotropic retrovirus (FTLV) that causes chronic immune suppression in infected cats. Clinical signs are generally manifest in middle age cats (4-7 years) that have harbored the virus for an extended period, but cats of any age may contract the disease. These signs may include chronic gingivitis, periodontal disease, chronic anemias and leukopenias, pustular dermatopathies, chronic upper respiratory syndrome, and generalized lymphadenopathy. FIV is infectious and contagious by blood cell exposure, usually through bite wounds. The presence of FIV antibodies in a cat indicates exposure and, due to the nature of the lentivirus, implies persistent infection.

ViraCHEK® FIV uses a highly specific FIV peptide to quickly identify antibodies to FIV in infected cats. ViraCHEK® FIV has been optimized to use whole blood, plasma or serum specimens. The test takes 15 minutes to detect circulating antibodies to FIV even before clinical signs of infection are apparent.

II. TEST PRINCIPLE

The plastic wells are coated with Protein A, an antibody-capturing protein. A highly specific peptide of FIV is labeled with horseradish peroxidase (HRP). The specimen (either feline whole blood, plasma or serum) is incubated simultaneously with the coated wells and enzyme-labeled FIV peptide. Antibodies to FIV, if present in the sample, are bound to the well and enzyme-linked FIV peptide at the same time. The free enzyme-linked peptide is washed away and a chromogenic substrate is added. The development of a distinctly blue color indicates the presence of antibody to FIV. In the absence of FIV antibody, no color change will be observed.

ViraCHEK® FIV is highly specific, sensitive and simple to perform. Test results can be obtained in 15 minutes. The diagnostic kit contains a positive control and a negative control which should be included each time the assay is performed. Visual comparison of the color of samples to the positive and negative controls will allow accurate detection of the presence of FIV antibody in the sample.

III. CONTENTS

Protein A Coated Wells	8 x 12
Bottle A HRP FIV peptide Conjugate	2 x 10.0 ml
Bottle B FIV Ab positive control	2.0 ml
Bottle C FIV Ab negative control	2.0 ml
Bottle D Substrate TMB blue	10 ml
Bottle W 10 x Wash Concentrate	200 ml

Additional material provided:

Wells holder
Hermetic Plastic pouch (place the plate with red dessicant in the pouch after first use)

Additional material required:

Micropipette
Deionized or distilled water
Squirt bottle
Timer

Optional:

Plate reader
Automatic plate washer

IV. PRECAUTIONS

1. Allow kit to come to room temperature (21°-25°C ; 70°-78° F) prior to use.
2. Do not expose kit to direct sunlight.
3. Do not use expired reagents or mix from different kit lots.
4. Follow instructions exactly. Improper washing or contamination of reagents may produce non-specific color development.

5. It is possible to distribute with a micropipette the HRP FIV peptide conjugate (Bottle A) and the substrate TMB blue (Bottle D). For that, remove the dropper of the bottles. Volumes to dispense are indicated between brackets in the test procedure (page 2). Properly close the bottles well to avoid any escape.

6. FOR VETERINARY USE ONLY.

V. SAMPLE INFORMATION

10 microliter (0.01 ml) of either whole blood (anti-coagulated with EDTA, heparin, etc.), plasma or serum is required. Use only feline samples for test specimens. Samples may be stored at 2-7°C up to seven days. If longer storage is desired, serum or plasma may be stored at -20°C. Severely hemolyzed or lipemic samples may produce background color. When in doubt, obtain a better quality sample.

For a better reliability of the results, it is strongly recommended not to test more than 10 samples per series of determination (because of time necessary for the distribution of the samples).

For a number of samples higher than 10, it is recommended to carry out the distribution of the samples using a multichannel micropipette starting from a plate of pre-dilution.

VI. PREPARATION OF WASH SOLUTION

Allow wash concentrate to come to room temperature. Mix gently by inversion.

Dilute wash concentrate 10-fold (1:10) with deionized or distilled water (e.g. 10 ml wash concentrate to 90 ml water).

The diluted wash solution can be stored 5 days at room temperature.

VII. RESULTS

1. For the test to be valid, the fluid in the positive control well must be distinctly blue, while that in the negative control well should show no color change from initial substrate color.
2. A color change in the test sample of greater intensity than the negative control indicates that the cat has been exposed to FIV and, due to the nature of the lentivirus, implies persistent infection. A negative test result indicates that the cat is free of the virus or has not yet sero-converted after exposure. Cats may sero-convert in as little as 3 weeks or as long as 10 months after becoming infected with FIV. Care must be taken when interpreting test results in kittens under four months of age as maternal FIV antibodies may be present.

VIII. STORAGE AND STABILITY

Store the test kit and unused diluted wash solution at 2°-7°C (36°C-45° F). Do not freeze. Reagents should be stable until expiration date provided they have been stored properly.

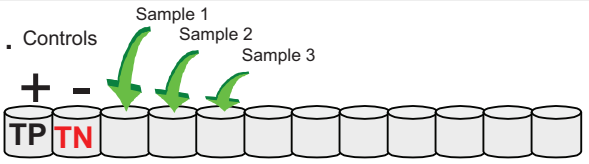
IX. REFERENCES

1. Pedersen NC, Ho EW, et al. Science 1987;235:790-793
2. Yamamoto JK, Sparger E, Ho EW, et al. Am J Vet Res 1988;49:1246-1258
3. Barr M. Feline Health Topics for Veterinarians 1988;3(3) 1-3,8
4. Pedersen NC: JAVMA 194(2):213-220,1989
5. Barr M., Pough MB Jacobson RH, Scott FW. Comparison and Interpretation of Diagnostic Tests for FIV. Proc. Of FeLV/FIV Colloquium, 1991.

ViraCHEK[®] FIV TEST PROCEDURE

A. SET UP AND CONJUGATE

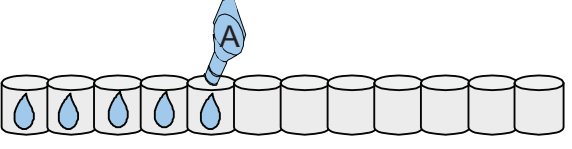
1. Controls



- Remove and place in holder:
 - 1 well for positive control (+)
 - 1 well for negative control (-)
 - 1 well for each sample.

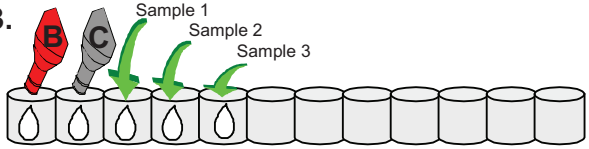
Leave the wells attached to each other.

2.



- Place 4 drops (160µl) of HRP FIV conjugate (Bottle A – Blue Cap) into each well.

3.



- Place 1 drop (40µl) of positive control (Bottle B – Red Cap) into the first well;
- Follow with 1 drop (40µl) of negative control (Bottle C – Green Cap) into the second well.
- Add 10 µl of sample to appropriate wells using a micropipette. Change tip between each sample
- Tap holder several times (10-15 sec).
- Incubation: **10 minutes at room temperature (70°F- 78°F) (21°C – 25°C)**
(if several specimens are run simultaneous, only one set of controls is needed)

B. WASHING

4.

Manual washing:

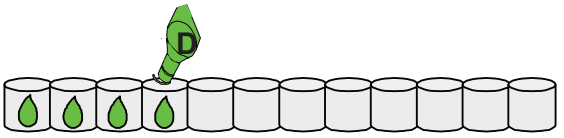
- empty the wells by inverting the holder and blotting onto a paper towel.
- wash the wells by filling them with the washing bottle to the edge with the solution of diluted washing buffer.
- empty the wells by inverting the holder and blotting onto a paper towel. **Repeat washing five (5) times**
- rinse once with distilled water in order to remove the bubbles, then invert the holder onto a paper towel.

Automatic washing:

- carry out a washing of 6 cycles with a washer of microplates ELISA. Think of filling open spaces of the holder with worn strips.

C. REVELATION

5.



- Place 2 drops (80µl) of substrate TMB blue (Bottle D – White Cap). Mix by gently tapping the holder several times
- Incubation: 5 minutes.**
- After incubation, slightly tap the holder during 5 seconds.
- Read results immediately (visual or spectrometer). See interpretation of the Results Section

D. RESULTS

6.

- Visual reading:**
The test is valid if the well of the positive control is blue and the negative control remains colorless.

* Any sample presenting a coloring lower or identical to the negative control is **negative**.

* Any sample presenting a coloring stronger than the negative control is **positive**.

- Spectrometer reading:**
Measure the optical densities of the samples in double wavelength 630 nm - 450 nm.
The test is validated if the positive control has a value superior to 0,150

* Any sample having an optical density lower or equal to 0,020 (or the optical density of the negative control if this one is higher than 0,020) is **negative**.

* Any sample having an optical density higher than 0,020 (or with the optical density of the negative control if this one is higher than 0,020) is **positive**.

ATTENTION: The results should be interpreted immediately after the 5 minutes incubation period. Prolonged incubation may result in non-specific color development.

GOOD TECHNIQUES = ACCURATE RESULTS

- Whole blood must be anti -coagulated with EDTA, heparin, citrate, etc.
- Hemolyzed and lipemic samples may be used however, severely hemolyzed and lipemic samples may produce background colour. When in doubt, obtain a better quality sample.
- Washing is the most important step. Microwells cannot be overwashed. Underwashing will result in colour development in the negative control and sample wells.
- Prolonged incubation for more than 5 minutes in step 6 may result in non-specific colour development. Read results at 5 minutes. If no colour is seen at 5 minutes, the sample is negative.
- Always compare results to the positive and negative controls.
- Do not use the test kit past the expiration date and do not intermix components from different serial numbers.
- Store kit at 2°-7° C (36° -45° F).
- Allow kit to come to room temperature before use.

Should you have any question, please contact us :
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