

EIA FPA

EQUINE INFECTIOUS ANEMIA ANTIBODY TEST KIT, FPA

The EIA FPA TEST KIT is a qualitative test using Fluorescence Polarization technology designed to determine the presence of antibodies to Equine Infectious Anemia Virus. The presence of the virus is indicative of prior infection with EIAV. The EIA FPA test is validated for testing serum samples of horses.

The diagnostic test uses a peptide conjugate as tracer. A fluorescence polarization instrument is used to measure the polarization state of the light emitted by the conjugate. When no antibodies are present, the polarization is low. Polarization increases when antibodies bind to the conjugate.

EIA FPA is a homogenous assay with the entire reaction in liquid phase and no washing steps.

Kit Contents

Reagents	250 tests	1000 tests
Positive Control	1 ml	1 ml
<p>Ready-to-use; equine positive serum against Equine Infectious Anemia Virus. Contains 0.095% sodium azide as a preservative. Hazard Code: Not classified according to EU regulations.</p>		
Negative Control	2 ml	2 ml
<p>Ready-to-use; equine negative serum against Equine Infectious Anemia Virus. Contains 0.095% sodium azide as a preservative. Hazard Code: Not classified according to EU regulations.</p>		
10X Sample Diluent	50 ml	2 x 50 ml
<p>Proprietary formula: Sample Diluent is a mixture of non-hazardous substances dissolved in ultrapure water. Hazard Code: Not classified according to EU regulations.</p>		
Tracer	2.5 ml	10 ml
<p>Ready-to-use; proprietary formula that contains peptide labeled with fluorescein. Contains 0.095% sodium azide as a preservative. Hazard Code: Not classified according to EU regulations.</p>		

Materials Required But Not Provided

- An FP instrument
- 10 x 75 or 12 x 75 mm borosilicate glass test tubes for tube instruments
- Black 96 well microtiter plates for microplate instrument, Product Code: PLATES96
- 4Tititude 24 well Assay Plate, solid bottom, black, Product Code: PLATES24
- Precision single micropipettes and tips for volumes between 10 to 1000 μ l (e.g., single pipettes 10-100 and 100-1000 μ l)
- Distilled or deionized water
- Plastic or glass bottles with screw caps and laboratory beakers or Erlenmeyer flasks to make ready-to-use Sample Diluent

For supplies, contact our customer support at support@ellielab.com

Storage & Stability

The kit should be stored at 2-8 °C.

During use, avoid exposing the Negative Control to temperatures higher than room temperature (up to 25°C).

The kit is transported in a cooled box at temperatures between 0 and 15°C.

Warnings

- All reagents are for *in vitro* diagnostic use only.
- Do not pipette by mouth.
- Avoid contact with open skin.
- Avoid pipetting that creates bubbles.
- Polarization readings are affected by temperature; all reagents used in the test should be at the same temperature as the samples being tested. Avoid temperature variations during testing.
- Sodium azide is a toxic substance, and it is used in some reagents. In case of contact with eyes or skin, flush immediately with copious amounts of water. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposing of reagents, flush with a large volume of water to help prevent azide build-up.
- Instruments used to read test results must be obtained from or approved by Ellie LLC. Warranty or performance is not guaranteed otherwise.

All materials in this kit should be treated according to the product Safety Data Sheet.

Specimen Requirements

Ellie's EIA FPA test can be used with serum samples from horses.

The test uses only 40 µl of serum for duplicate tests. Collect the amount of blood required by the blood collection system.

Collect blood aseptically in untreated tubes or serum separator tubes. Allow the blood to clot and separate the serum. Fresh sera should be used for testing. Store sera at 2-8 °C for 7 days or freeze it at -20 °C if not tested within 72 hours; avoid repeated freezing.

Hemolyzed, lipemic and lyophilized serum samples are acceptable for testing.

Preliminary Steps

Ensure the Sample Diluent is free of particulates. Heat up to 37°C to dissolve any crystals. Then, equilibrate to room temperature before use.

Prepare Sample Diluent by mixing one part 10X Sample Diluent with 9 parts of distilled or deionized water.

Lyophilized serum samples should be reconstituted completely and frozen samples fully thawed and mixed.

Lipemic serum samples (non-transparent serums) and samples with free particles must be centrifuged for 5 minutes at 13 000 rpm.

Testing Procedure

1. Pipette 20 µl of each sample and control into tubes suitable for the Sentry tube instrument or wells of a microtiter plate for the Sentry microplate instrument. Run Negative Controls in triplicate. Avoid bubbles when pipetting into strips or microtiter plates.

NOTE: When testing with tubes, retest the controls after every 60 samples.

2. Pipette 1 ml of the Sample Diluent into all tubes, 24 well Assay plate or pipette 180 µl of Sample Diluent into all wells of the 96 well microtiter plate. Mix carefully.
3. Incubate for 4-6 minutes at room temperature.
4. Obtain blank readings for all samples and controls.
5. Add 10 µl of Tracer into all tubes/wells containing controls and samples. Mix carefully.
6. Incubate for 4-6 minutes at room temperature.
7. Obtain mP readings for all samples and controls.

Test Validation

1. The Negative Control must read between 70 and 95 mP.
2. The Positive Control must read between 120 and 250 mP.
3. If the Negative Control is outside of the above range, adjust the instrument to read the mean Negative Control at 80 ±5 mP. For further instructions, consult the instrument manual. Depending on the instrument, this can be done without retesting samples.
4. If the Negative Control is adjusted and the Positive Control is outside of the above range, the test is considered invalid. Please contact technical support at support@ellielab.com.

Results & Interpretation

Calculation of ΔmP values

Calculate ΔmP values by subtracting the mean Negative Control mP value from the sample mP value:

$$\Delta mP = (\text{Sample mP} - \text{Average Negative Control mP})$$

Interpretation:

Negative ≤ 10	Suspect 10-20	Positive >20
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Positive and suspect samples must be retested in duplicate. If both retests read equal or less than 10 ΔmP , the sample is reported as Negative. If any of the retests are higher than 10 ΔmP , the sample is reported as Positive.

Quality Control

Upon the first use of the test kit, record the ΔmP of the Positive Control. Also, record the mP value of the Negative Control. This information should be systematically recorded and followed. The ΔmP of the Positive Control is a true indication of the condition of the test kit and the instrument. The mP of the Negative Control is an indication of the testing condition and the condition of the Negative Control.

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