

EQUINE INFECTIOUS ANEMIA VIRUS ANTIBODY TEST KIT, ELISA

ViraCHEK™ | EIA

ENGLISH

For the detection of Equine Infectious Anemia Virus (EIA) antibodies in equine serum.

GENERAL INFORMATION AND INTENDED USES

ViraCHEK™ EIA uses a highly purified recombinant antigen to quickly identify antibodies to EIA in infected equines without causing the non-specific reactions commonly found in cultured antigen ELISA tests. ViraCHEK™ EIA has been optimized to use serum specimens. The correlation between ViraCHEK™ EIA and LAB-E™ EIA is greater than 99%.

The plastic wells are coated with EIA recombinant antigen. The same EIA recombinant antigen is labeled with horseradish peroxidase (HRP). The specimen (serum) is incubated simultaneously with the coated wells and enzyme-labeled antigen. Antibodies to EIA, if present in the equine sample, are bound to the well and enzyme-linked antigen at the same time. The free enzyme-linked antigen is washed away and a chromogenic substrate is added. The development of a distinctly dark blue color indicates the presence of antibody to EIA. In the absence of EIA antibody, little or no color change will be observed.

ViraCHEK™ EIA is highly specific, sensitive and simple to perform. Test results can be obtained in 20 minutes or less. The diagnostic kit contains a POSITIVE CONTROL and a NEGATIVE CONTROL that MUST be included each time the assay is performed. Visual comparison of the color of the sample to the POSITIVE CONTROL will allow accurate detection of the presence of EIA antibody in the sample. If desired, test results may be determined by use of a microwell plate reader.

KIT COMPOSITION AND CONSERVATION

Contains materials sufficient to test 5 - 24 samples.

| ITEM | REAGENT NATURE | VOLUME | RECONSTITUTION AND CONSERVATION |
|----------|--|----------------------|--|
| M | EIA Antigen Coated Wells | 2 sets of 4x12 wells | Ready to use |
| CONTROL+ | Positive Control; preserved with Phenol and Gentamicin sulfate | 1.6 mL | Ready to use. Red Cap. |
| CONTROL- | Negative Control; preserved with Phenol and Gentamicin sulfate | 1.6 mL | Ready to use. Gray Cap. |
| A | Conjugate; preserved with Phenol and Gentamicin sulfate | 5.0 mL | Ready to use. Blue Cap. |
| D | Chromogen | 7.5 mL | Ready to use. Green Cap. |
| E | Substrate Buffer; preserved with Sodium Benzoate | 7.5 mL | Ready to use. White Cap. |
| F | 10X Wash Concentrate; preserved with Gentamicin sulfate. | 100 mL | Dilute to 1X in deionized or distilled water. Orange Cap. Diluted Wash Solution may be stored at 2 - 7 °C. |
| | Well holder | | Ready to use. |

Store all reagents provided in the kit at 2 - 7 °C. Reagents should not be frozen.

REAGENTS REQUIRED TO PERFORM 24 TESTS

- a) 2 sets of 4x12 EIA Antigen Coated Wells
- b) 1.6 mL Positive Control
- c) 1.6 mL Negative Control
- d) 5.0 mL Conjugate
- e) 7.5 mL Chromogen
- f) 7.5 mL Substrate Buffer
- g) 100 mL 10X Wash
- h) Well holder

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- a) 50 µL pipette
- b) Disposable pipette tips
- c) Deionized/distilled water
- d) 2 Wash Bottles
- e) Timer
- f) Microplate reader (optional)

WARNINGS TO THE USERS OF REAGENTS AND ANTIGEN COATED MICROPLATES

- Handle all reagents and samples as biohazardous material. It is recommended to dispose reagents and contaminated material according to the applicable regulations.
- Wear suitable protective clothing.
- Irritating to skin and eyes. Keep all reagents away from skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Take care not to contaminate any test reagents with samples or bacterial agents.
- The best results are achieved by following the protocols described below, using good, safe laboratory techniques.
- Do not use this kit or any of its contents after the expiration date.
- Do not intermix components from different serial numbers.
- Use a separate pipette tip for each sample.
- Follow instructions exactly. Improper washing or contamination of reagents may produce nonspecific color development.
- Do not expose kit to direct sunlight.
- NEVER PIPETTE BY MOUTH. Harmful if swallowed.

SAMPLE COLLECTION AND STORAGE

- Follow proper sample collection procedures.
- Harvest serum samples and store properly (up to seven days at 4 °C; -20 °C for longer).
- Test only good quality samples (i.e. avoid bacterial contamination, heavy hemolysis or lipemia). When in doubt, obtain a better quality sample.

Allow all reagents to come to 21 - 24 °C before starting. PREPARATION OF WASH SOLUTION

Allow 10X wash concentrate to come to ambient temperature. Mix gently by inversion. Dilute wash concentrate 10-fold with distilled or deionized water (1 part concentrate to 9 parts deionized or distilled water) in a wash bottle. Diluted wash solution may be stored at 2 - 7 °C.

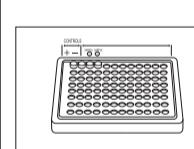
TEST PROCEDURE

STEP

NOTES

SET UP AND SAMPLE INCUBATION

- 1) Remove and place in holder one well for Positive Control, one well for Negative Control, and one well for each sample. Leave the wells attached to each other.

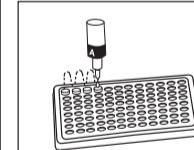


NOTE:

- When testing a high number of samples in an assay, Zoetis strongly recommends including one Negative Control well and one Positive Control well for every 22 samples tested within a run.
- If a microplate reader will be used to read the results, leave the appropriate space empty so that the microplate reader will blank on air.

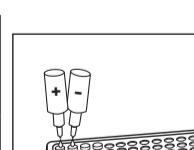
CONJUGATE

- 2) Add 1 drop of Conjugate (Bottle A - Blue Cap) into each well.



SAMPLE ADDITION

- 3) Add 1 drop of Positive Control (Red Cap) into the first well.



- 4) Add 1 drop of Negative Control (Gray Cap) into the second well.

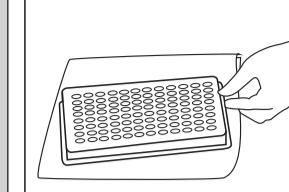


- 5) Pipette 50 µL (0.05 mL) of sample into the next well following the controls. Repeat for each additional sample into subsequent wells. One well is used for each sample. Gently tap the well holder (without splashing) for 15 seconds to mix.

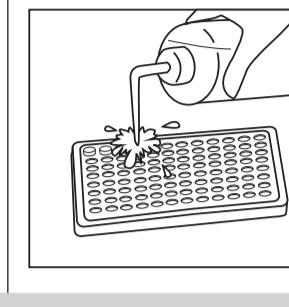
- 6) Incubate for 10 minutes.

BLOT AND WASH

- 7) Discard the fluid from wells into appropriate container. Invert holder and blot firmly onto a paper towel to remove final drops.



- 8) FLUSH WELLS VIGOROUSLY:
 - Wash by vigorously filling the wells to overflowing with diluted wash solution.
 - Direct a forceful stream into each well. (Oversplashing will not contaminate adjacent wells).
 - Shake out excess water.
Repeat wash cycle five (5) times.

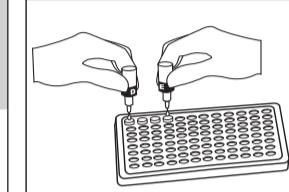


- 9) Wash wells 2 more times with distilled or deionized water to remove bubbles.

- 10) Blot against a paper towel to dry wells.

DEVELOP

- 7) Add 1 drop of Chromogen (Bottle D - Green Cap) into each well.



- 8) Add 1 drop of Substrate Buffer (Bottle E - White Cap) into each well. Tap well holder (without splashing) for 15 seconds to mix.

- 9) Incubate for 10 minutes.

- 10) Read results at exactly 10 minutes.

INTERPRETATION OF RESULTS

Controls:

- **POSITIVE** control should be distinctly blue.
- **NEGATIVE** control should be completely clear.

Samples:

- **POSITIVE** samples will be blue. Color intensity (optical density) will be equal to or greater than that of the **POSITIVE CONTROL**.
- **NEGATIVE** samples will produce a color intensity (optical density) lower than that of the **POSITIVE CONTROL**. Compare directly with the positive control against a white background.

NOTES

- Only serum may be used as a sample.
- **Washing is the most important step.** Wells cannot be overwashed. Underwashing will result in nonspecific blue color development in the negative control and sample wells.
- Read results at 10 minutes. If no color is seen at 10 minutes, the sample is negative.
- Always compare results to the Positive Control. Wells can be detached and compared alongside the Positive Control well against a white background for easier visual inspection.

SYMBOL DESCRIPTIONS



Use by Date (expiration date)



EC REP Authorized Representative in the European Community



LOT Batch Code



IVD Consult Instructions for Use



SN Serial Number



IVD In Vitro diagnostic medical device



Manufacturer

zoetis

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VLN/PCN 190/5515.00



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