

Precautions

Kit components should be handled and disposed of as potentially hazardous. Do not eat, drink, or smoke where serum samples and kit reagents are handled. Do not pipette by mouth. Some reagents may be harmful if ingested. If ingested, seek medical attention. Do not use expired or contaminated reagents, or reagents from other kits or serials. Do not mix reagents from different serials of this same product.

Component B, Positive Control, contains ProClin 300 as a preservative.

Component C, Negative Control, contains ProClin 300 as a preservative.

Component D, 100X Antigen-Peroxidase Conjugate, contains ProClin 300, methylisothiazolone, bromonitrodioxane, and thimerosal as preservatives.

USDA Veterinary License No. 332

Version 130920

EQUINE INFECTIOUS ANEMIA VIRUS ANTIBODY TEST KIT, ELISA

Assay instructions for catalog numbers: 290-1 and 290-5

General Description

This enzyme-linked, immunosorbent assay (ELISA) detects antibodies to Equine Infectious Anemia Virus (EIAV) in equine sera. Sample serum EIAV antibodies bind recombinant EIAV p26 antigen coated on the plastic wells. Non-specific antibody is washed away and plate-bound EIAV-specific antibody captures the HRP-labeled recombinant p26 protein conjugate via some free Fab antigen binding sites. Unbound conjugate is washed away and the presence of bound HRP-labeled conjugate is detected by the addition of an enzyme substrate with subsequent blue color product development. The addition of stop solution slows the enzyme reaction and changes the color product from blue to yellow. A cutoff positive control provides a color reference for visually reading results as well as an optical density (OD) reference for reading the assay with a microplate absorbance spectrophotometer. Yellow color or OD equal to or greater than the positive control indicates the presence of antibodies to EIAV p26 in sample sera. Color or OD lower than the positive control indicates the absence of detectable antibodies to EIAV p26.

Kit Contents

Component	290-1	290-5
A Antigen-Coated Plates	1 plate	5 plates
B Positive Control	2 ml	4 ml
C Negative Control	2 ml	4 ml
D 100X Antigen-Peroxidase Conjugate	0.15 ml	0.5 ml
E Conjugate Diluting Buffer	15 ml	60 ml
F 10X Wash Solution Concentrate	60 ml	2 x 120 ml
G Substrate Solution	15 ml	60 ml
H Stop Solution	15 ml	60 ml
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Materials Required But Not Included in the Test Kit

Single and multichannel adjustable-volume pipettors and disposable plastic tips, test tubes or non-antigen-coated transfer plate(s), ELISA microplate absorbance spectrophotometer with 450 nm filter, deionized or distilled water, paper towels, graduated cylinder, timer, multichannel pipettor reservoirs, wash bottle, manual multichannel washing device or automatic plate washer

Storage and Stability

Store all reagents at 2-7°C. **Do not freeze.** Unopened reagents will remain stable until the expiration date when stored as instructed. **Do not use test kit past the expiration date printed on the box.**

Preparation

- a. **Warm reagents:** Bring the serum samples, reagents and plate(s) to room temperature ($23 \pm 2^\circ\text{C}$) prior to starting the test.
- b. **Prepare controls and samples:** Positive and Negative Controls are provided ready to use. When whole plates are used, it is best to put the controls in wells on different areas of the plate. Controls must be loaded on every plate. Serum samples are tested UNDILUTED.
- c. **Prepare plates:** Remove the plate(s) from the foil pouch(es) (A). If applicable: Return any unused strips to the pouch and securely seal it. Extra pouches and sealer are available from VMRD. Place strips to be used in the frame and number the top of each strip to maintain orientation. Always mark the strips in case they dislodge from the frame during washing.
- d. **Prepare conjugate:** Prepare 1X Antigen-Peroxidase Conjugate by diluting 1 part of the 100X Antigen-Peroxidase Conjugate (D) with 99 parts of Conjugate Diluting Buffer (E). Example: For 96 wells, mix 60 μl of 100X Antigen-Peroxidase Conjugate (D) with 5.940 ml of Conjugate Diluting Buffer (E) to yield 6 ml of 1X Antigen-Peroxidase Conjugate. Fifty microliters (50 μl) are needed per well. Diluted (1X) Antigen-Peroxidase Conjugate is stable for two weeks when stored at 2-7°C.
- e. **Prepare wash solution:** Prepare 1X Wash Solution by diluting 1 part of the 10X Wash Solution Concentrate (F) with 9 parts of deionized or distilled water. Approximately 1 ml is needed per well. Allow extra quantity for reservoirs, tubing, pipetting, etc. Diluted (1X) Wash Solution is stable for two weeks when stored at 2-7°C.

Test Procedure

1. **Load controls and serum samples:** Using a pipettor set at 50 μl , load controls and serum samples into the Antigen-Coated Plate (A). Serum samples and controls should be loaded into the Antigen-Coated Plate (A) as quickly as possible. When running more than two strips, we recommend that the serum samples and controls be first loaded into a transfer plate and then transferred to the Antigen-Coated Plate (A) using multi-channel pipetting equipment. The sample volume in the transfer plate must be in excess of 50 μl in order to transfer 50 μl from it. Tap the side of the loaded assay plate several times to make sure the samples coat the bottom of the wells. Use care not to spill samples from well to well. Incubate the plate for 10 minutes at room temperature ($23 \pm 2^\circ\text{C}$).
2. **Wash wells:** After the 10-minute incubation wash the plate one time: *If an automatic washer is used*, place the plate on the washing apparatus and wash plate 1 time, filling the wells each time with 1X Wash Solution. *If manual*

washing is used, dump well contents and remove remaining sera and controls by sharply striking the inverted plate 4 times on a clean paper towel, striking a clean area each time. Immediately fill each well with 1X Wash Solution using a multichannel filling device or a wash bottle. Empty the wash solution from the plate and strike the inverted plate sharply on a clean paper towel as above.

3. **Add antigen-peroxidase conjugate:** Add 50 μl of diluted (1X) Antigen-Peroxidase Conjugate to each well. Tap the side of the loaded assay plate several times to make sure the conjugate coats the bottom of the wells. Incubate for 10 minutes at room temperature ($23 \pm 2^\circ\text{C}$).
4. **Wash wells:** After the incubation, wash the plate 4 times as in Step 2.
5. **Add substrate solution:** Add 50 μl of Substrate Solution (G) to each well. Tap the side of the loaded assay plate several times to make sure the substrate coats the bottom of the wells. Incubate 15 minutes at room temperature ($23 \pm 2^\circ\text{C}$). Avoid leaving the plate in direct sunlight. *Do not empty wells.*
6. **Add stop solution:** Add 50 μl of Stop Solution (H) to each well. When Stop Solution is added, the color will turn from blue to yellow. Tap the side of the loaded assay plate several times to mix the Substrate Solution and the Stop Solution. *Do not empty wells.*
7. **Read and record the test results:** Immediately after adding the Stop Solution, the plate should be observed visually against the Positive Control or should be read on a microplate absorbance spectrophotometer. Set the optical density (O.D.) reading wavelength to 450 nm. Blank the instrument on air and read plate(s). Some readers require an empty well on the plate for blanking. In this case, no reagents should be added to this well.
8. Return all remaining kit reagents to 2-7°C for storage.

Interpreting the Results

- **Microplate reader interpretation:** Samples having an O.D. greater than or equal to the O.D. of the Positive Control are positive for antibody to EIAV p26. Samples having an O.D. less than the Positive Control should be considered negative. For the test to be valid, the O.D. of the Positive Control should be greater than or equal to 1.5 times the O.D. of the Negative Control. The O.D. of the Negative Control should be less than or equal to 0.15.
- **Visual interpretation:** Samples with the same or greater color than the Positive Control are positive. Samples with no color or faint color less than the Positive Control are negative. For the test to be valid, the Positive Control should have visible yellow color and the Negative Control should have no or faint visible color that is less than the Positive Control.

Positive results should be verified by AGID. In the event of discrepant results, the sample should be sent to the National Veterinary Services Laboratories (NVSL) for confirmation.