



# EQUINE INFECTIOUS ANEMIA VIRUS ANTIBODY TEST KIT, ELISA

Assay instructions for catalog numbers: 290-1, 290-2 and 290-5

USDA Product Code 5515.00 • For veterinary use only

## General Description

This enzyme-linked immunosorbent assay (ELISA) detects serum antibody to Equine Infectious Anemia Virus (EIAV). The kit uses recombinant EIAV p26 Antigen-Coated Plates. Equine serum is incubated in the Antigen-Coated Plates and removed by dumping. Bound antibody is detected by adding Antigen-Peroxidase Conjugate (purified recombinant EIAV p26 antigen; the same antigen used to coat wells) followed by washing and Substrate Solution addition. After incubation, Stop Solution is added, changing the color of the Positive Control and any positive serum samples from blue to yellow. The results can be read using a microplate reader or visually by comparison with the Positive Control. This test for antibodies to EIAV is rapid, yet provides high specificity and sensitivity.

## Kit Contents

<b>Component</b>	<b>290-1</b>	<b>290-2</b>	<b>290-5</b>
<b>A</b> Antigen-Coated Plates	1 plate	2 plates	5 plates
<b>B</b> Positive Control	2 ml	4 ml	4 ml
<b>C</b> Negative Control	2 ml	4 ml	4 ml
<b>D</b> 100X Antigen-Peroxidase Conjugate	150 µl	300 µl	500 µl
<b>E</b> Conjugate Diluting Buffer	15 ml	30 ml	60 ml
<b>F</b> 10X Wash Solution Concentrate	60 ml	120 ml	2 x 120 ml
<b>G</b> Substrate Solution	15 ml	30 ml	60 ml
<b>H</b> Stop Solution	15 ml	30 ml	60 ml

This insert with Setup Record for recording sample identifications and results.

## 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 reader or spectrophotometer with 450 nm filter (optional). Deionized or distilled water. Paper towels. Multichannel pipettor reservoirs. Graduated cylinder. Manual multichannel washing device or automatic plate washer. Timer.

## Storage and Stability

Store all reagents at 2-7°C (35-45°F). **Do not freeze.** 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.**

## Precautions

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. Do not mix reagents from different lots of this product.

## Preparation

- a. **Warm up reagents:** Bring the serum samples, reagents and plate(s) to room temperature prior to starting the test.
- b. **Position controls and samples:** Use Positive and Negative Controls with each run. When testing a large number of samples, it is recommended that the samples first be placed in a non-antigen-coated transfer plate and transferred to the antigen-coated plate with a multichannel micropipettor. When whole plates are used, it is best to put the controls in wells on different parts of the plate. Controls must be run on every plate. Enter the control and serum sample IDs on a photocopy of the attached Setup Record.
- c. **Prepare plates:** Remove the plate(s) from the foil pouch(es) (**A**). If any of the rings on the humidity card have turned pink, the integrity of the plate may have been compromised. If so, please call VMRD. *If applicable:* Return any unused strips to the pouch and securely seal it. Call VMRD for extra pouches and sealer. Place strips to be used in the frame and number the top of each strip to maintain orientation with the Setup Record. Always mark the strips in case they fall out of 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**). Fifty microliters (50  $\mu$ l) are needed per well. *Example:* For 96 wells, mix 60  $\mu$ l of 100X Antigen-Peroxidase Conjugate (**D**) with 5.940 ml of Conjugate Diluting Buffer (**E**) to yield 6 ml of 1X Antigen-Peroxidase Conjugate. 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 one 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.
- f. **Prepare serum samples:** Serum samples are tested UNDILUTED.

## Test Procedure

1. **Load controls and serum samples:** Using a pipettor set at 50  $\mu$ l, transfer controls and serum samples to the Antigen-Coated Plate (**A**) according to the Setup Record. 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 (21-25°C, 70-77°F).
2. **Wash wells:** After the 10-minute incubation wash the plate one time:  
*If manual washing is used*, dump contents of the wells into a sink and remove the remaining sera and controls by sharply striking the inverted plate four times on a clean paper towel, striking a clean area each time. Immediately fill each well with 1X Wash Solution using a repeating syringe with a manifold, multichannel pipettor, or multichannel washing device. Dump out the Wash Solution and strike the inverted plate sharply on a clean paper towel as above.

If an automatic washer is used, place the plate on the washing apparatus and wash plate once, aspirating and then filling the wells with 1X Wash Solution.

- 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 Antigen-Peroxidase Conjugate coats the bottom of the wells. Incubate for an additional 10 minutes at room temperature (21-25°C; 70-77°F).
- Wash wells:** After the 10-minute Antigen-Peroxidase Conjugate incubation, wash the plate four times as described in Step 2.
- 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 Solution coats the bottom of the wells. Incubate 15 minutes at room temperature (21-25°C; 70-77°F). Avoid leaving the plate in direct sunlight. *Do not empty wells.*
- 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.*
- Read and record the test result:** Immediately after adding the Stop Solution, the plate should be observed visually against the Positive Control or should be read on a plate reader. Set the optical density (O.D.) reading wavelength to 450 nm. Blank reader 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.
- Return all remaining kit reagents to 2-7°C (35-45°F) 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.

**U.S. Veterinary License No. 332**

Version 060509



# SETUP RECORD

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Date: \_\_\_\_\_ Case ID: \_\_\_\_\_ Kit Serial: \_\_\_\_\_ Catalog Nos.: 290-1, 290-2, 290-5