

SETUP RECORD

|    |   |   |   |   |   |   |   |   |
|----|---|---|---|---|---|---|---|---|
| 12 |   |   |   |   |   |   |   |   |
| 11 |   |   |   |   |   |   |   |   |
| 10 |   |   |   |   |   |   |   |   |
| 9  |   |   |   |   |   |   |   |   |
| 8  |   |   |   |   |   |   |   |   |
| 7  |   |   |   |   |   |   |   |   |
| 6  |   |   |   |   |   |   |   |   |
| 5  |   |   |   |   |   |   |   |   |
| 4  |   |   |   |   |   |   |   |   |
| 3  |   |   |   |   |   |   |   |   |
| 2  |   |   |   |   |   |   |   |   |
| 1  |   |   |   |   |   |   |   |   |
|    | A | B | C | D | E | F | G | H |

Catalog Nos.: 289-2 and 289-5

Kit Serial: \_\_\_\_\_

Case ID: \_\_\_\_\_

Date: \_\_\_\_\_



**CAPRINE ARTHRITIS-ENCEPHALITIS VIRUS  
ANTIBODY TEST KIT cELISA**

**Assay instructions for catalog numbers: 289-2 and 289-5**

USDA Product Code 5139.20 • For veterinary use only

**General Description**

This competitive enzyme-linked immunosorbent assay (cELISA) detects antibodies to caprine arthritis encephalitis virus (CAEV) in goat sera. Sample serum CAEV antibody inhibits binding of horseradish peroxidase (HRP)-labeled CAEV-specific monoclonal antibody to CAEV antigen coated on the plastic wells. Binding of the HRP-labeled monoclonal antibody conjugate is detected by the addition of enzyme substrate and quantified by subsequent color product development. Strong color development indicates little or no blockage of HRP-labeled monoclonal antibody binding and therefore the absence of CAEV antibody in sample sera. Weak color development due to inhibition of the monoclonal antibody binding to the antigen on the solid phase indicates the presence of CAEV antibodies in sample sera.

**Kit Contents**

| <b>Component</b>                            | <b>289-2</b> | <b>289-5</b> |
|---|--------------|--------------|
| <b>A</b> Antigen-Coated Plates              | 2 plates     | 5 plates     |
| <b>B</b> Positive Control                   | 3.6 ml       | 3.6 ml       |
| <b>C</b> Negative Control                   | 3.6 ml       | 3.6 ml       |
| <b>D</b> 100X Antibody-Peroxidase Conjugate | 300 µl       | 500 µl       |
| <b>E</b> Conjugate Diluting Buffer          | 30 ml        | 60 ml        |
| <b>F</b> 10X Wash Solution Concentrate      | 120 ml       | 2 x 120 ml   |
| <b>G</b> Substrate Solution                 | 30 ml        | 60 ml        |
| <b>H</b> Stop Solution                      | 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 620, 630 or 650 nm filter. Deionized or distilled water. Paper towels. Multichannel pipettor reservoirs. Graduated cylinders. Wash bottle, 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 contain ProClin 300, sodium azide or sodium fluoride. These chemicals may be harmful if ingested. If ingested, seek medical attention. Material Safety Data Sheet (MSDS) is available on request or through our web site: <http://www.vmr.com>. Do not use expired or contaminated reagents, or reagents from other kits. Do not mix reagents from different lots of this same 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:** Run both the Positive Control (**B**) and Negative Control (**C**) in duplicate, regardless of the number of serum samples to be tested. 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 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 Antibody-Peroxidase Conjugate by diluting one part of the 100X Antibody-Peroxidase Conjugate (**D**) with 99 parts of Conjugate Diluting buffer (**E**). Example: For 96 wells, mix 60 µl of Antibody-Peroxidase Conjugate (**D**) with 5.940 ml of Conjugate Diluting Buffer (**E**) to yield 6 ml of ready-to-use Antibody-Peroxidase Conjugate. Fifty microliters (50 µl) are needed per well. Allow extra quantity for reservoirs, tubing, pipetting, etc.
- 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 1.5 ml are needed per well. Allow extra quantity for reservoirs, tubing, pipetting, etc.
- f. **Prepare serum samples:** Serum samples are tested UNDILUTED.

## Test Procedure

1. **Load controls and serum samples:** Using a pipettor set at 50 µl, 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 1 hour at room temperature (21-25°C, 70-77°F).
2. **Wash wells:** After the 1-hour incubation, wash the plate three times:  
*If an automatic washer is used,* place the plate on the washing apparatus and wash plate three times, filling the wells each time with 1X Wash Solution.  
*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 repeating syringe with a manifold, wash bottle or multichannel pipettor. Dump out the Wash Solution and strike the inverted plate sharply on a clean paper towel as above. Repeat the washing procedure two more times (three washes total).
3. **Add conjugate:** Add 50 µl of diluted Antibody-Peroxidase Conjugate (**D**) 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 an additional 30 minutes at room temperature (21-25°C, 70-77°F).

4. **Wash wells:** After the 30-minute incubation, repeat the washing procedure described in Step 2 (3 washes total).
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 20 minutes at room temperature (21-25°C; 70-77°F). 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. Gently mix the well contents by tapping the side of the plate several times. *Do not empty wells.*
7. **Read and record the test result:** Immediately after adding the Stop Solution, the plate should be read on a plate reader. Set the optical density (O.D.) reading wavelength to 620, 630 or 650 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.
8. Return all remaining kit reagents to 2-7°C (35-45°F) for storage.

## Test Validation

- The mean of the **Negative Controls** must produce an optical density  $\geq 0.300$ .
- The mean of the **Positive Controls** must produce  $\geq 35\%$  inhibition.

Calculation of percent inhibition (% I):

$$\% I = 100 - [(Sample\ O.D. \times 100) \div (Mean\ Negative\ Control\ O.D.)]$$

## Interpreting the Results

- If a test sample produces  $\geq 35\%$  inhibition, it is positive.
- If a test sample produces  $< 35\%$  inhibition, it is negative.

## U.S. Veterinary License No. 332

Version 070125

