

ELITEST – MVV/CAEV # CK104A

Kit for the detection of antibodies to Maedi-Visna virus (MVV) in sheep serum and Caprine Arthritis Encephalitis Virus (CAEV) in goat serum

For in vitro use only

For research use only

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INTENDED USE

ELITEST MVV/CAEV is an Enzyme ImmunoAssay (EIA) for the detection of antibodies to Maedi Visna Virus (MVV) in sheep serum and Caprine Arthritis Encephalitis Virus (CAEV) in goat serum.

CLINICAL BACKGROUND

MVV and CAEV cause a slowly evolving disease of sheep or goat characterized by interstitial pneumonia, meningoencephalitis, indurative mastitis, and non suppurative arthritis. The infection has been reported to be of high prevalence in many countries but only some of the infected animals develop chronic progressive lesions and eventually die from the infection. The etiological agent is the prototype of the *Lentivirinae* sub-family of the *Retroviridae*, and are collectively known as small ruminant lentiviruses (SRLV). The lentiviruses have the ability to evade the defense mechanisms of their natural host, thus causing persistent infection. The infected animals remain carriers of the virus for life, contributing to the propagation of the infection within flocks. Viral transmission occurs principally between the dam and her progeny, via the colostrum and milk. The degree of transmission between the dam and her offspring is high.

In order to control the spread of the virus, the viral carriers have to be detected and eradicated as no vaccine or treatment is yet available. As most of the infected sheep or goat develop a humoral response, the demonstration of anti-virus antibodies by serological techniques is indicative of viral infection.

TEST PRINCIPLE

The wells of polystyrene microplate strips have been coated with a synthetic peptide and a recombinant protein derived from MVV envelope and gag proteins, respectively. These coated proteins and peptides constitute the solid phase antigens. The test sample is incubated in the well and viral specific antibodies to the envelope and/or gag protein, if present in the sample, will bind to the solid phase antigens.

Subsequently, an affinity-purified rabbit anti-sheep IgG (H + L chain) labelled with the enzyme horseradish peroxidase (HRP), is added (second antibody). Upon a positive reaction, this labelled antibody will bind to the antigen/antibody complex previously formed on the solid phase. Subsequent incubation with substrate produces a blue colour in the test well, which turns into yellow when the reaction is stopped with sulfuric acid. If the sample contains no antibodies, the labelled antibody will not bind specifically, and only a low background colour develops.

REAGENTS SUPPLIED

Each pack contains:

- 5 sachets containing a strip-holder with 12 x 8 **MVV-coated test wells** (synthetic peptide and recombinant protein, representing the envelope and gag antigens of MVV, respectively). A silicagel bag is added as desiccant.
- 1 vial containing 125 µl of **negative control (NC)** (sheep serum containing 0.1% sodium azide as preservative), and 1 vial containing 125 µl of **positive control (PC)** (sheep serum containing 0.1% sodium azide as preservative).
- 1 vial containing 250 ml of **Sample Diluent (SD)**: phosphate buffer containing sodium chloride, Triton⁽¹⁾ protein stabilizers and 0.05 % Kathon CG as preservative).
- 1 vial containing 75 ml of **Conjugate Diluent (CD)**: phosphate buffer containing protein and enzyme stabilizers and 0.05 % Kathon CG as preservative).
- 1 vial containing 0.75 ml of concentrated **Conjugate (C)**: rabbit anti-sheep IgG (H + L chain) labelled with horseradish peroxidase, to be diluted 1:100 before use.
- 1 vial containing 0.75 ml of concentrated **TMB substrate solution (S)**: tetramethylbenzidine dissolved in dimethyl sulfoxide, to be diluted 100 x before use.
- 1 vial containing 75 ml of **Substrate Buffer (SB)**: phosphate citrate buffer containing 0.006% hydrogen peroxide).
- 1 vial containing 150 ml of concentrated **Wash Solution (WS)**: phosphate buffer containing 1.25 % Tween 20) to be diluted 1:25 before use.

- 1 vial containing 75 ml of **stop solution (SS)** sulfuric acid)
- 15 adhesive plate sealers**.
- 1 plastic minigrip bag** storage of unused strips.

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water.
- Precision pipettes with disposable tip to deliver in the ranges 1-10µl, 20-200µl; and 200-100µl, respectively.
- Optionally a multichannel pipette to deliver 100 µl can be used together with disposable V-shaped troughs for addition of conjugate, substrate and sulfuric acid.
- Microplate shaker (optional).
- Incubator set at 37°C.
- Microplate washer (alternatively, washing can be performed manually, e.g. by using a repeating syringe delivering 0.3 ml volumes and an aspirating device).
- Absorbent tissues.
- Photometric reading: microplate reader, equipped with a 450 nm filter and also with 595 nm filter (or alternatively 690 nm instead 595 nm).

Safety: Avoid contact with and inhalation of TMB solution. If substrate comes into contact with skin, wash thoroughly with water.

STORAGE AND STABILITY

- If kept at 2° to 8°C, all test reagents, including the coated test wells, are stable until the expiration date given on the pack.
- All reagents and the sachet containing the test wells must be brought to room temperature (15-30°C) approximately 30 minutes before use and must be returned to the refrigerator immediately after use.
- Unused test wells, stored at 2-8°C, are stable for 8 weeks if stored in the plastic minigrip bag with silicagel. Moisture adversely affects test performance. **Hence, when the silicagel turns red, discard the remaining strips.**
- Diluted wash solution is stable for 2 weeks, if kept at 2-8°C.
- Diluted conjugate is stable for 4 hours at room temperature (15-30°C) if kept in the dark.
- Diluted substrate is stable for 1 hour at room temperature (15-30°C) if kept in the dark.
- After using some of the contents of vials containing controls, sample diluent, concentrated conjugate, conjugate diluent, concentrated substrate, substrate buffer, and concentrated wash solution, the contents are stable until the expiration date if kept at 2-8°C and stored in the closed original vial.

SPECIMENS

- ELITEST MVV/CAEV may be performed on sheep and goat serum.
- Specimens should be free of microbial contamination when tested.
- Additives give erroneous results.
- Insoluble material should be removed from all samples by centrifugation before testing.
- Before storage, serum should be separated from blood clot or blood cells by centrifugation. For storage longer than one week, freeze in aliquots at -20° C.
- Store the samples at 2-8 °C. For storage longer than one week, freeze in aliquots at -20°C.

TEST PERFORMANCE

Preparations:

- Allow all test material to reach room temperature (15-30°C) before use.
- Washing solution should be prepared by diluting concentrated wash solution (WS) (8) 1:25 with distilled water or deionized water, e.g. by diluting 20 ml to 500 ml. Prepare at least 50 ml of diluted wash solution for each strip.

Note: Salt crystals may be formed in the concentrated wash solution after storage at 2-8°C. These crystals should be completely redissolved, by warming at 37°C, before dilution.

Wash solution must be at room temperature (15-30°C) when used.

- Make a **1:100 predilution** of your samples and controls e.g. 4 µl serum in 400 µl sample diluent.
- Conjugate should be prepared by diluting concentrated conjugate (C) (5) 1:100 with conjugate diluent (CD) (4), e.g. by diluting 10µl to 1 ml per strip or 100 µl to 10 ml per plate.
- Substrate should be prepared by diluting concentrated TMB substrate (S) (6) 1:100 with substrate buffer (SB) (7), e.g. by diluting 10 µl to 1 ml per strip or 100 µl to 10 ml per plate.

Note: Concentrated TMB should be melted completely (melt. point 18°C)

Directions for washing:

Incomplete washing will adversely affect the test outcome. Operating instructions for washing equipment should be carefully followed. Contamination of wash solution and washer can cause extensive problems.

Therefore: - Store diluted wash solution at 2-8°C. Preferably use freshly prepared wash solution.
- Prerinse the washer with diluted wash solution.

In case problems occur, disinfect wash bottles and washer overnight with 4% formaldehyde solution.

If no suitable automatic washer is available, washing can be performed manually as follows: aspirate completely the liquid from all wells by lowering an aspiration tip gently to the bottom of each well. **Take care not to scratch the inside of the well surface.** After aspiration, fill the wells with diluted wash solution ensuring that the liquid reaches to the top of the wells. Let soak for a minimum of 30 seconds, then aspirate the liquid. Perform these steps five times. After the last aspiration, the washing procedure is completed by inverting the plate and tapping it dry on absorbent tissue.

Remarks and precautions:

1. Do not use the kit beyond the expiration date.
2. Do not mix reagents between kits, unless the components have identical lot numbers.
3. All vessels used to prepare conjugate and substrate solutions must be cleaned thoroughly and finally rinsed with distilled water.
4. Do not touch the top of the plates with your fingers to avoid contamination.
5. Avoid microbial contamination of reagents.
6. Ensure that the samples and controls are homogeneous solutions before use.
7. Use a new pipette-tip for each specimen aliquoted.
8. To avoid contamination, do not touch the edges of the wells with the pipette tips when adding sample or conjugate.
9. Do not expose substrate to strong light during incubation or storage. Substrate solution must be almost colourless when used.
10. Solutions containing TMB, sulfuric acid or peroxide should not be in contact with metals or metal-ions, to avoid unwanted colour formation.
11. Make sure no air-bubbles in the wells are present; remove any bubbles by tapping gently.
12. If the wells cannot be filled with conjugate or substrate immediately after washing, the strips may be placed upside down on a wet absorbent tissue for no longer than 15 minutes.

ASSAY PROCEDURE

Before starting the assay, adjust the temperature of the incubator to 37°C.

1. Fill the strip-holder with the required number of strips, taking into account that one negative control and one positive control should be included. If more strips are used in one run, at least two negative and positive controls should be included in each strip holder. During the processing, strips stay in the strip holder and can be marked on one edge.
2. Make a 1:100 predilution of your samples and controls e.g. 4 µl serum in 400 µl sample diluent. For milk samples use a dilution of 1:10. Do not use very small volumes of serum (e.g. 1 µl or less) to avoid pipetting errors.

Reagent addition:

3. Add 80 µl of diluent to each test well.
4. Add 20 µl of diluted sample or control to each appropriate test well. Make sure specimen and control are adequately and completely mixed with the diluent on a microplate shaker (# 1000 rpm) or manually by pipetting up and down (at least five times).
5. Cover the strips with an adhesive sealer. Incubate for 60 minutes at 37 °C.

(Note: prepare conjugate solution during incubation, see Preparations).

6. Wash each well 5 times (see Directions for washing).
7. Add 100 µl prediluted conjugate solution to each well.
8. Cover the strips with a new adhesive sealer. Incubate for 60 minutes at 37°C.

Note: prepare substrate solution during incubation, see Preparations.

9. Wash each well 5 times (see Directions for washing).
10. Add 100 µl prediluted substrate solution to each well.
11. Incubate for 30 minutes at 20-25°C.
12. To stop the reaction, add 100 µl stop solution to each well, in the same sequence and at the same time intervals as the substrate solution. Tap the strip holder carefully to ensure thorough mixing.
13. Read (within 15 minutes after step 12) the absorbance of the solution in the wells at 450 nm; 595 nm should be used as reference wavelength

SUMMARY OF ASSAY PROCEDURE

Reagent	Volume	
Sample diluent (Ready to use)	100 µl	Add to one test well, reserved as blank.
	80 µl	Add to each test well, reserved for specimen and controls.
Diluted samples or control	20 µl (prediluted 1/100)	Add to each appropriate test well, mix on plate or manually by pipetting up and down, cover plate with adhesive sealer.
Incubate for 60 minutes at 37°C		
Warning : prepare conjugate before the end of incubation		
Wash solution (to be diluted 25x before use in <u>distilled deionized water</u>)	5 x 0.3 ml	Wash each well 5 times.
Conjugate (to be diluted 100x before use in <u>conjugate diluent</u>)	100 µl	Add to each well, mix gently. Cover plate with new adhesive sealer.
Incubate for 60 minutes at 37°C		
Warning : prepare substrate solution before the end of incubation		
Wash solution (to be diluted 25x before use in <u>distilled or deionized water</u>)	5 x 0.3 ml	Wash each well 5 times.
Substrate (to be diluted 100x before use in <u>substrate buffer</u>)	100 µl	Add to each well
Incubate for 30 minutes at 20-25°C		
Stop solution	100 µl	Add to each well. Mix by tapping side of plate
Read absorbance at 450 nm – 595 nm within 15 minutes.		

INTERPRETATION

Abbreviations:

- P = the mean of the absorbance of the positive controls
- N = the mean of the absorbance of the negative controls
- S = the mean of the absorbance of the test sample

Validation:

Check the validity of individual negative and positive controls (absorbance at 450-595 nm)

- Each of the negative controls should be ≤ 0.100.
- Calculate N excluding controls out of the range specified.
- Each of the positive controls should be within the range indicated on the flyer provided with the kit.
- Calculate P excluding controls of the range specified.

If more than half of the controls have to be eliminated, the test run should be repeated after careful investigation into the source of possible errors.

Test result:

Calculate the cut-off value as: (P-N)/4 + N

A sample is NON-REACTIVE if S < (P-N)/4 + N

A sample is REACTIVE if S ≥ (P-N)/4 + N