



BABESIA EQUI ANTIBODY TEST KIT, cELISA

Assay instructions for catalog numbers: 274-2 and 274-5

USDA Product Code 501B.20 • For veterinary use only

General Description

This competitive enzyme-linked immunosorbent assay (cELISA) detects *B. equi* antibodies in equine sera. Sample serum *B. equi* antibody inhibits binding of primary monoclonal antibody. The binding of primary monoclonal antibody to the antigen-coated plate is detected with HRP-labeled secondary antibody. Finally, the presence of HRP-labeled secondary antibody is quantified by the addition of enzyme substrate and subsequent color product development. Strong color development indicates little or no inhibition of primary monoclonal antibody binding and therefore the absence of *B. equi* antibody in sample sera. Weak color development due to inhibition of the primary monoclonal antibody binding to the antigen on the solid phase indicates the presence of *B. equi* antibodies in sample sera.

Kit Contents

Component	274-2	274-5
A Antigen-Coated Plates	2 plates	5 plates
B Positive Control	2 ml	4 ml
C Negative Control	2 ml	4 ml
D 100X Primary Antibody	300 µl	500 µl
E 100X Secondary Antibody-Peroxidase Conjugate	300 µl	500 µl
F Antibody Diluting Buffer	60 ml	120 ml
G Serum Diluting Buffer	9 ml	25 ml
H 10X Wash Solution Concentrate	120 ml	2 x 120 ml
I Substrate Solution	30 ml	60 ml
J 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 cylinder. 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 or sodium fluoride. These chemicals 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 same product.

Preparation

- a. **Warm up reagents:** Bring the serum samples, reagents and plate(s) to room temperature prior to starting the test.
- b. **Prepare controls and samples:** The Positive and Negative Controls (**B & C**) and test serum samples must be diluted 1:2 with Serum Diluting Buffer (**G**) for use in the test. It is recommended that these dilutions be made in a non-antigen-coated transfer plate. Run Positive Control (**B**) in duplicate and Negative Control (**C**) in triplicate, 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 primary antibody:** Prepare 1X Primary Antibody by diluting 1 part of the 100X Primary Antibody (**D**) with 99 parts of Antibody Diluting Buffer (**F**). Example: For 96 wells, mix 60 µl of 100X Primary Antibody (**D**) with 5.940 ml of Antibody Diluting Buffer (**F**) to yield 6 ml of 1X Primary Antibody. Fifty microliters (50 µl) are needed per well.
- e. **Prepare secondary antibody-peroxidase conjugate:** Prepare 1X Secondary Antibody-Peroxidase Conjugate by diluting 1 part of the 100X Secondary Antibody-Peroxidase Conjugate (**E**) with 99 parts of Antibody Diluting Buffer (**F**). Example: For 96 wells, mix 60 µl of 100X Secondary Antibody-Peroxidase Conjugate (**E**) with 5.940 ml of Antibody Diluting Buffer (**F**) to yield 6 ml of 1X Secondary Antibody-Peroxidase Conjugate. Fifty microliters (50 µl) are needed per well.
- f. **Prepare wash solution:** Prepare 1X Wash Solution by diluting 1 part of the 10X Wash Solution Concentrate (**H**) with 9 parts of deionized or distilled water. Approximately 1.8 ml are needed per well. Allow extra quantity for reservoirs, tubing, pipetting, etc.

Test Procedure

1. **Load controls and serum samples:** Using a pipettor set at 50 µl, transfer diluted 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 30 minutes at room temperature (21-25°C, 70-77°F).
2. **Wash wells:** After the 30-minute incubation, wash the plate 3 times:

If an automatic washer is used, place the plate on the washing apparatus and wash plate 3 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 4 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 2 more times (3 washes total).

3. **Add primary antibody:** Add 50 µl of diluted (1X) Primary Antibody to each well. Tap the side of the loaded assay plate several times to make sure the primary antibody 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 second 30-minute incubation, wash the plate 3 times as in Step 2.
5. **Add secondary antibody-peroxidase conjugate:** Add 50 µl of diluted (1X) Secondary Antibody-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 an additional 30 minutes at room temperature (21-25°C, 70-77°F).
6. **Wash wells:** After the third 30-minute incubation, wash the plate 3 times as in Step 2.
7. **Add substrate solution:** Add 50 µl of Substrate Solution (**I**) 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 (21-25°C, 70-77°F). Avoid leaving the plate in direct sunlight. *Do not empty wells.*
8. **Add stop solution:** Add 50 µl of Stop Solution (**J**) to each well. Tap the side of the loaded assay plate several times to mix the Substrate Solution and the Stop Solution. *Do not empty wells.*
9. **Read and record the test results:** 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 the 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.
10. 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 >0.300 and <2.000.
- The mean of the **Positive Controls** must produce an inhibition of ≥40%.

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 ≥ 40% inhibition, it is positive.
- If a test sample produces <40% inhibition, it is negative.



SETUP RECORD

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11												
12												
	A	B	C	D	E	F	G	H				

Date: _____ Case ID: _____ Kit Serial: _____ Catalog Nos.: 274-2 and 274-5