

Interpreting the Results

- Test samples having < 30% inhibition are negative.
- Test samples having ≥ 30% inhibition are positive.

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 sodium azide as a preservative.

Component C, Negative Control, contains sodium azide as a preservative.

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

Component E, Conjugate Diluting Buffer, contains ProClin 300 as a preservative.

Component H, Stop Solution, contains sodium fluoride.

USDA Veterinary License No. 332

Version 160412

ANAPLASMA ANTIBODY TEST KIT, cELISA v2

Assay instructions for catalog number: 283-2

General Description

This *Anaplasma* Antibody Test Kit, cELISA, is a competitive, enzyme-linked, immunosorbent assay (cELISA) for the detection of antibodies specific for *Anaplasma* in bovine serum samples. It is intended to provide results which will give guidance about the presence of *Anaplasma* infection in bovine species. The principle of the test is as follows: Sample serum antibodies to *Anaplasma* inhibit the binding of a horseradish peroxidase (HRP)-labeled monoclonal antibody to the *Anaplasma* antigen coated on the plastic wells. Binding, or lack of 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 antibodies to *Anaplasma* in the sample serum. Weak or no color development due to inhibition of the monoclonal antibody binding to the antigen on the solid phase indicates the presence of *Anaplasma* antibodies in the sample serum.

Kit Contents

Component	
A Antigen-Coated Plates	2 plates
B Positive Control	3.6 ml
C Negative Control	3.6 ml
D 100X Antibody-Peroxidase Conjugate	0.3 ml
E Conjugate Diluting Buffer	30 ml
F 10X Wash Solution Concentrate	120 ml
G Substrate Solution	30 ml
H Stop Solution	30 ml
This insert	

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 620, 630 or 650 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.** 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:** Load 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 areas of the plate. Controls must be loaded on every plate.
Serum samples and controls 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 Antibody-Peroxidase Conjugate by diluting 1 part of the 100X Antibody-Peroxidase Conjugate (D) with 99 parts of Conjugate Diluting Buffer (E). Example: For 96 wells, mix 60 μl of 100X Antibody-Peroxidase Conjugate (D) with 5.940 ml of Conjugate Diluting Buffer (E) to yield 6 ml of 1X Antibody-Peroxidase Conjugate. Fifty microliters (50 μl) are needed per well.
- 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 1.5 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 controls and serum samples to 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 1 hour at room temperature ($23 \pm 2^\circ\text{C}$).

2. **Wash wells:** After the 1-hour incubation, wash the plate 2 times:

If an automatic washer is used, place the plate on the washing apparatus and wash the plate 2 times, 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. Fill and empty the plate by the same method 1 additional time for a total of 2 washes.

3. **Add conjugate:** Add 50 μl of diluted (1X) 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 20 minutes at room temperature ($23 \pm 2^\circ\text{C}$).
4. **Wash wells:** After the 20-minute incubation, wash the plate 4 times as described 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 for 20 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. 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 read on a microplate absorbance spectrophotometer. Set the optical density (OD) reading wavelength to 620, 630 or 650 nm 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.

Calculation of % Inhibition (% I):

$$\% I = 100 [1 - (\text{Sample OD} \div \text{Negative Control OD})]$$

Test Validation

- The mean of the **Negative Controls** must have an optical density > 0.40 and ≤ 2.10 .
- The mean of the **Positive Controls** must have an inhibition of $\geq 30\%$.