## Calculation of % Inhibition (% I):

% I = 100 [1-(Sample O.D. ÷ NC O.D.)]

## 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 ≥ 30%.

## 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 C, Positive Control, contains sodium azide as a preservative.

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

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

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

Component H, Stop Solution, contains sodium fluoride.

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**FOR VETERINARY USE ONLY**

USDA Product Code 5002.20

# *ANAPLASMA* ANTIBODY TEST KIT, cELISA

Assay instructions for catalog numbers: 282-2 and 282-5

## 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 that 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 | 282-2 | 282-5 |
|---|---|---|
| A Antigen-Coated Plates | 2 plates | 5 plates |
| B Coated Adsorption/Transfer Plates | 2 plates | 5 plates |
| C Positive Control | 3.6 ml | 3.6 ml |
| D Negative Control | 3.6 ml | 3.6 ml |
| E 100X Antibody-Peroxidase Conjugate | 0.3 ml | 0.5 ml |
| F Conjugate Diluting Buffer | 30 ml | 60 ml |
| G 10X Wash Solution Concentrate | 120 ml | 2 x 120 ml |
| H Substrate Solution | 30 ml | 60 ml |
| I Stop Solution | 30 ml | 60 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 plates to room temperature (23 ± 2°C) prior to starting the test.

b. **Position controls and samples:** Load Positive Control (C) in duplicate and Negative Control (D) 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 are tested UNDILUTED.

c. **Prepare plates:** Remove the Anaplasma Antigen-Coated Plate (A) and the Coated Adsorption/Transfer Plate (B) from their pouches. 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 Concentrate (E) with 99 parts of Conjugate Diluting Buffer (F). Example: For 96 wells, mix 60 µl of 100X Antibody-Peroxidase Conjugate Concentrate (E) with 5.940 ml of Conjugate Diluting Buffer (F) 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 (G) 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 70 µl, load controls and serum samples into the Coated Adsorption/Transfer Plate (B). Serum samples and controls should be loaded into the Coated Adsorption/Transfer Plate (B) 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 Coated Adsorption/Transfer Plate (B) using multi-channel pipetting equipment. The sample volume in the transfer plate must be in excess of 70 µl in order to transfer 70 µl from it. Tap the side of the loaded 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 for 30 minutes at room temperature (23 ± 2°C). Then, using a single or multichannel pipettor, transfer 50 µl of the adsorbed serum test samples to the corresponding wells of the Anaplasma Antigen-Coated Plate (A). 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 the samples from well to well. Incubate the plate 1 hour at room temperature (23 ± 2°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 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 times 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 an additional 20 minutes at room temperature (23 ± 2°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 (H) 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 (23 ± 2°C). Avoid leaving the plate in direct sunlight. *Do not empty wells.*
6. **Add stop solution:** Add 50 µl of Stop Solution (I) 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 (O.D.) reading wavelength to 620, 630 or 650 nm. Blank the instrument 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 for storage.

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