



ANAPLASMA ANTIBODY TEST KIT, cELISA

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

USDA Product Code 5002.20 • For veterinary use only

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	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 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. 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, sodium azide or sodium fluoride. These chemicals may be harmful if ingested. If ingested, seek medical attention. A 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 plates to room temperature (21-25°C, 70-77°F) prior to starting the test.
- b. **Position controls and samples:** Make copies of the attached Setup Record. Enter the sample identifications on the Setup Record and label the Coated Adsorption/Transfer Plate (**B**) and the *Anaplasma* Antigen-Coated Plate (**A**) carefully according to the Setup Record for the samples to be tested. Run the Positive Control (**C**) in duplicate and the 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 parts of the plate. Controls must be run on every plate.
- 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. 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 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. 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 (**G**) with nine 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 70 µl, transfer controls and serum samples to the Coated Adsorption/Transfer Plate (**B**) according to the Setup Record. 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 (21-25°C, 70-77°F). 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 serum 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 two times:

If an automatic washer is used, place the plate on the washing apparatus and wash plate two 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 one more time (two washes total).

3. **Add conjugate:** Add 50 µl of diluted 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 (21-25°C, 70-77°F).
4. **Wash wells:** After the 20-minute incubation, wash four times.
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 (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 (**I**) 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 O.D. of the **Negative Control** must range from 0.40 to 2.10.
- The percent inhibition of the **Positive Control** must be ≥30%.

Calculation of % inhibition (% I):

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

Interpreting the Results

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

U.S. Veterinary License No. 332

Version 060505



SETUP RECORD

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3												
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8												
9												
10												
11												
12												
	A	B	C	D	E	F	G	H				

Date: _____ Case ID: _____ Kit Serial: _____ Catalog Nos.: 282-2, 282-5