



## **Anaplasma / Rickettsiales ELISA Kit**

Enzyme Immunoassay for the determination of Anaplasma / Rickettsiales in dog serum.

Catalog number: ARG83060

Package: 96 wells

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For research use only. Not for use in diagnostic procedures.

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### INTRODUCTION

Anaplasma is a genus of bacteria of the alphaproteobacterial order Rickettsiales, family Anaplasmataceae. Anaplasma species reside in host blood cells and lead to the disease anaplasmosis. The disease most commonly occurs in areas where competent tick vectors are indigenous, including tropical and semitropical areas of the world for intraerythrocytic Anaplasma spp. Anaplasma species are biologically transmitted by Ixodes deer-tick vectors, and the prototypical species, *A. marginale*, can be mechanically transmitted by biting flies and iatrogenically with blood-contaminated instruments. One of the major consequences of infection by bovine red blood cells by *A. marginale* is the development of nonhaemolytic anaemia, thus the absence of hemoglobinuria, which allows clinical differentiation from another major tick-borne disease, bovine babesiosis, caused by *Babesia bigemina*.

### PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique. A specific Anaplasma / Rickettsiales antigen has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Anaplasma / Rickettsiales antibody present is bound by the immobilized antigen. After washing away any unbound substances, an HRP-conjugated antibody is added to each well and incubate. Following the washing of any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of antigen-antibody binding in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm  $\pm$ 2nm.

### MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Calibrator A (Negative Control)	2ml (Ready-to-use)	4°C
Calibrator B (Cut-off Standard)	3ml (Ready-to-use)	4°C
Calibrator C (Positive Control)	2ml (Ready-to-use)	4°C
HRP-Streptavidin conjugate	20 ml (Ready-to-use)	4°C
Sample Diluent Buffer	100 ml (Ready-to-use)	4°C
20X Wash Buffer	50 ml	4°C
TMB Substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP Solution	15 ml (Ready-to-use)	4°C
Plate sealer	1 e.a.	Room temperature

### MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (Optional: 620 nm as reference wavelength)
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

### TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times and do not use after the expiry date.
- It is very important to bring all reagents and samples to room temperature (20-25°C) and mix them before starting the test run.
- Unused strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2-8 °C.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved. Mix well before dilution.
- The TMB Color developing agent should be colorless (or could have a slight blue tinge) and transparent before using.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- All materials should be equilibrated to room temperature (RT; 20-25°C) before use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Perform all assay steps in the order given and without any delays.
- Change pipette tips between the addition of different reagent or samples.
- For further internal quality control each laboratory should additionally use known samples.

### SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Serum**- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at -20 or -70°C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using citrate and heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20 or -70°C. Avoid repeated freeze-thaw cycles.

Note:

If samples are frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

### REAGENT PREPARATION

- **1X Wash buffer:** Dilute **20X** Wash buffer into **distilled water** to yield 1X Wash buffer. (e.g. 10 ml of 20X Wash buffer +190 ml of distilled water). Mix thoroughly by Use a magnetic stirrer. The diluted 1X wash buffer is stable for 5 days at room temperature (20-25 °C).
- **Patient sample:** Dilute patient sample **1:101** with **Sample Diluent buffer** before assay, mix well. (e.g. 5 µl of serum + 500 µl of Sample Diluent buffer)

**Note:** The controls are ready-to-use and need not further dilution.

### ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25 °C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **controls, diluted samples (1:101)** into wells. Leave one well empty for the Substrate Blank. Cover the wells and incubate for **1h at 37°C**.
3. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes** (for automatic systems wash the wells for a total 5 washes). Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Avoid overflows from the reaction wells. Keep the wash buffer in the wells for **> 5 sec** before removal. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

**Note:** If performing the test on ELISA automatic systems we recommend increasing the washing steps from **three** up to **five** and the volume of Washing Buffer from **300 µL** to **350 µL** to avoid washing effects.

4. Add **100 µl** of **HRP-conjugated antibody** into each well (except for the Substrate Blank well). Cover wells and incubate for **30 minutes at RT**. (Do not expose to direct sunlight)
5. Aspirate each well and **wash as step 3**.
6. Add **100 µl** of **TMB Substrate Reagent** to each well. Incubate for **15 minutes** at **room temperature** in dark. A blue color occurs due to an enzymatic reaction.

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7. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at **450 nm** (and reference filter **620 nm**) immediately. It is recommended read the absorbance within 30 minutes after adding the stop solution.

### CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Adjust the ELISA Microtiterplate reader to zero using the Substrate Blank.
3. Bichromatic measurement using a reference wavelength of 620 nm is recommended.
4. In order for an assay to be considered valid, the following criteria must be met:

Substrate blank: Absorbance value **< 0.100**

Negative control: Absorbance value **< 0.200** and **< cut-off**

Cut-off control: Absorbance value **0.150- 1.300**

Positive control: Absorbance value **> cut-off**

If these criteria are not met, the test is not valid and must be repeated.

5. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value of Cut-off Control well 1 = 0.5

absorbance value Cut-off control well 2 = 0.52

Control mean absorbance = Cut-off =  $(0.5+0.52)/2 = 0.51$



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6. Results in Units [U] (Ex.: If sample mean absorbance =1.2)

Units [U] = [Sample (mean) absorbance value x 10] / Cut-off

Example:  $(1.2 \times 10) / 0.51 = 23.5$  U (Units)

Note: Cut-off = (Cut-off X 10) / Cut-off = 10 U

7. Interpretation of results:

- The samples are considered positive if the absorbance value is higher than 10% over the cut-off.

- Samples with absorbance value of 10% above or below cut-off should be considered in the grey zone.

*It is recommended to repeat test again 2-4 weeks later with fresh sample. If the results in the second test are again in the grey zone, the sample has to be considered negative.*

- Samples are considered negative if the absorbance value is lower than 10% below the cut-off.

Summary:

	Unit	Note
Cut-off	10 U	-
Positive	> 11 U	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine)
Equivocal	9 – 11 U	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative.
Negative	< 9 U	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.

Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.

### **QUALITY ASSURANCE**

#### **Intra-assay and Inter-assay precision**

The CV values of intra-assay precision is 2.7% and inter-assay precision is 7.5%.

#### **Diagnostic Specificity**

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analytic.

It is 94.12% (95% confidence interval: 71.31%-99.85%).

#### **Diagnostic Sensitivity**

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analytic.

It is 92.00% (95% confidence interval: 73.97%- 99.02%)

#### **Cross Reactivity**

Cross-reactions cannot be excluded.

#### **Interferences**

Interferences are not significant interference effect observed up to concentration of 10 mg/mL hemoglobin, 15 mg/mL Triglycerides and 0.5 mg/mL bilirubin.