

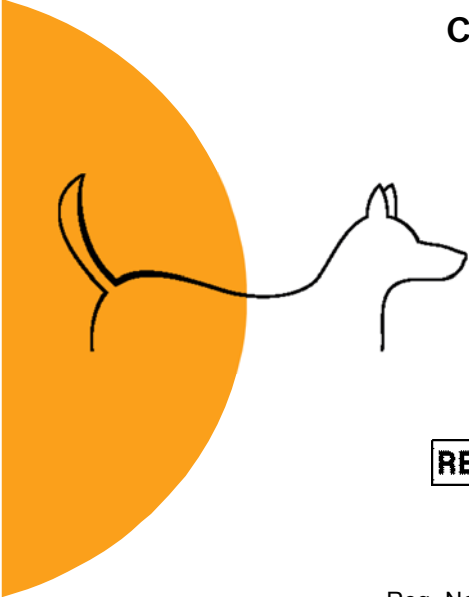
Instructions for use

ANAPLASMA-ELISA DOG

Test to detect the presence of antibodies against the cause of anaplasmosis, *Anaplasma phagocytophilum*


In-vitro Diagnostic for Dogs

Contents of the Kit:


- 
- 1 Microtitre plate, 96 wells
(coated with *Anaplasma*-antigen)
 - 50 ml Sample dilution buffer
 - 50 ml Wash buffer (10x concentrate)
 - 12 ml Conjugate solution
 - 12 ml Substrate solution
 - 12 ml Stop solution
 - 1.2 ml Positive control serum
 - 1.2 ml Negative control serum

REF AED-Kit

LOT
Lot number:
see sticker


Expiration date:
see sticker

Reg.-No.: FLI-B 678

 2...8°C



AFOSA GmbH • Ludwig-Erhard-Ring 3 • D-15827 Blankenfelde-Mahlow • Germany
Tel.: 033708-9381 85 • Fax: 033708-9381 86 • info@afosa.com • www.afosa.de

- **Fast and simple testing**
- **Ready-to-use reagents**

PRODUCT DESCRIPTION

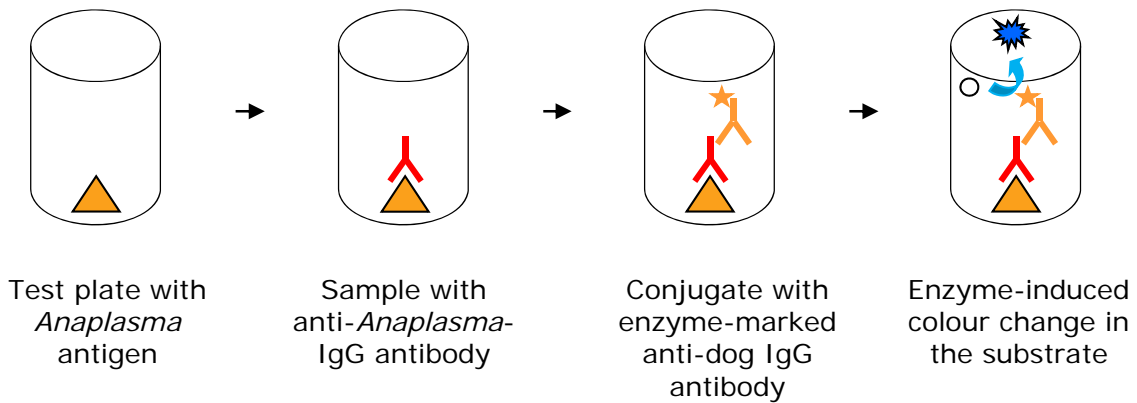
ANAPLASMA-ELISA DOG is an *in-vitro* diagnostic test to detect the presence of IgG antibodies against *Anaplasma phagocytophilum*, the pathogen of the anaplasmosis, in samples of dog serum.

GENERAL INFORMATION

The pathogen of dog anaplasmosis, *Anaplasma phagocytophilum*, is transmitted by blood feeding of *Ixodes ricinus* ticks. Host animals of the parasite, especially small rodents living in the wild, serve as a reservoir of Anaplasma bacteria. The pathogen is an obligate gram-negative intracellular bacterium that infests neutrophil granulocytes. Clinical symptoms of anaplasmosis in dogs are mostly unspecific such as high temperature, lethargy, anorexia, muscular and joint pain, splenomegaly as well as lymphadenomegaly. Mild to severe thrombocytopenia is a typical symptom of an acute anaplasmosis. Further symptoms include: lymphopenia, eosinopenia and mild to medium normochromic normocytic anaemia; the neutrophils often are in normal range. When there is suspicion due to clinical symptoms it is possible but difficult to find the "morulae" in the dyed blood smear. In most cases detection of antibodies is not possible during the acute stage of the disease. In general antibodies are detectable 10 to 14 days post infection, which is two to five days after morulae can be seen. A remarkable rise of antibodies within seven through 21 days is characteristic of an active Anaplasma infection. But a high-level antibody titre persisting for several months does not point out an acute anaplasmosis. If there is suspicion of anaplasmosis then besides the clinical symptoms the titre dynamic should be monitored. The sensitivity and the specificity of the Anaplasma-ELISA dog is nearly the same as known from the Anaplasma IFAT. Validation of the ELISA using 154 characterised sera from dogs resulted in a sensitivity of 92.5 and specificity up to 97%. Cross reactions to *Ehrlichia canis* antibodies in dog sera cannot be ruled out.

DESCRIPTION OF THE TEST

The testing system detects the presence of IgG antibodies against *Anaplasma phagocytophilum* in samples of dog serum. The microtitre plates are coated with a preparation of *Anaplasma* antigens. During the incubation period of the serum sample, antibodies against *Anaplasma phagocytophilum* form are bound to the antigen. Unbound material is washed away. The enzyme conjugate that is added then binds to the IgG antibody that is already bound to the antigen. Unbound enzyme conjugate is likewise washed away. An added substrate solution is stained by the enzyme that is bound to the antibody. The strength of the colouring reaction is correlated to the amount of anti-*Anaplasma* antibodies present in the sample. Diagnostic evaluation is made by comparing the extinction values of samples against controls.



TEST KIT CONTENTS

- The reagents in one test kit are sufficient for 91 evaluations.
- Microtitre plate (1 plate):
Coated with *Anaplasma*-antigen (inactive),
12 stripes with 8 wells each = 96 wells per plate (single wells can be detached and used).
- Sample diluent (1 bottle, 50 ml):
Buffer, preserved with sodium azide, ready-to-use.
- Washing solution concentrate (1 bottle, 50 ml):
Phosphate buffered, 10x concentration, preserved with ProClin 300.
- Positive control serum (1 bottle, 1.2 ml): **RED cap**
Serum of dogs infected with *Anaplasma phagocytophilum*, preserved with sodium azide, ready-to-use
- Negative control serum PC (1 bottle, 1.2 ml): **GREEN cap**
Serum of dogs not infected with *Anaplasma phagocytophilum*, preserved with sodium azide, ready-to-use
- Conjugate solution (1 bottle, 12 ml): **RED cap**
Horseradish peroxidase-conjugated anti-dog IgG immunoglobulins, ready-to-use, preserved with ProClin 300
- Substrate solution (1 bottle, 12 ml): **BLUE cap**
TMB solution, (TMB = 3,3',5,5'-tetramethylbenzidine), ready-to-use
- Stopping solution (1 bottle, 12 ml): **YELLOW cap**
Sulphuric acid, 1 mol/l, ready-to-use, Caution: Corrosive!
- Instructions for use

ADDITIONAL REQUIRED DEVICES AND MATERIALS (not included in test kit)

- Container for production of 1x washing buffer
- Distilled or purified water
- Precision pipettes
- Single-use pipette tips
- Single-use containers for diluting the samples
- Pipette reservoirs
- Absorbent pad, e.g., paper towels (recommended for wiping out the plate / strips after the wash processes)
- If required, empty frames for the required number of microtitre stripes
- Plates or foils to cover the microtitre plate
- Containers for the required amounts of conjugate and substrate solutions
- Vortex mixer
- Stopwatch
- Washer for microtitre plate or multi-channel pipette (300 µl)
- Photometer for microtitre plate with 450 nm filter

INSTRUCTIONS

1. Use of microtitre plates

One microtitre plate can test a maximum of 91 samples.

The microtitre plate is delivered in a re-sealable foil bag. The packaging also includes a dry bag with an indicator.

The microtitre is comprised of 12 stripes, each with 8 reaction wells. Only as many stripes as required for the number of samples (e.g., 2 stripes for 10 samples) should be removed and stored at room temperature (18° to 25°C) in a separate, well sealed bag. The stripes that are not yet needed should be stored at a constant temperature between 2° and 8°C.

It is critical to close the foil bag carefully each time after usage. A change in the colour of the contents of the dry bag from blue to bright red indicates high relative humidity in the foil bag; the dry bag should be replaced as required.

Microtitre stripes should not be re-used!

2. Preparation of the test reagents

The necessary test reagents should be brought to room temperature (18° to 25°C) before use. Prior to use they should also be thoroughly mixed by shaking the bottle or by stirring the small plastic tube.

Take the necessary amounts of conjugate solution and the substrate solution from the holder, place them in a separate container, and bring to room temperature. Conjugate solution and substrate solution that are not yet required should be stored at a constant temperature of 2° to 8°C.

The substrate and conjugate solutions should not be exposed to strong light. After a long period of cooling, the substrate solution can acquire a faint blue colouring as a result of spontaneous reactions. This faint colouring will disappear once the solution is warmed to room temperature.

Produce the required amount of washing buffer by diluting the required amount of 10x concentrate with distilled water in a 1:10 ratio (1 part concentrate to 9 parts water). If there are crystals in the washing buffer concentrate, they can be dissolved by carefully warming the concentrate. If you are dissolving the entire amount of washing buffer at once, make certain that any salts that have crystallized out do not remain in the original packaging. For one stripe on the microtitre plate, combine 27 ml of distilled water with 3 ml of washing buffer concentrate. This produces 30 ml of ready-to-use washing buffer.

After use, all remaining reagents should again be stored at 2° to 8°C.

Direct sunlight should be avoided during the testing procedures.

3. Test Preparation

For the test, blood serum or blood plasma that is fresh, has been kept in cool storage, or has been stored frozen and then thawed may be used. Samples that have been thawed should be thoroughly mixed before they are used.

Combine 5 µl of the sample with 495 µl of the sample dilution buffer (1:100). Mix the thinned solution thoroughly.

To prevent cross-contamination, the samples should not come into contact with the components of the test kit.

The control samples are ready for use and should not be diluted!

For each test, it is necessary to perform a positive, negative and null (only sample dilution buffer) control, to ensure correct test procedures and to check the stability of the reagents.

4. Test Procedure

The test must be performed in the following sequence and without any delays. For each step with a pipette, use a clean, new single-use pipette tip.

- When in use, all reagents should be at room temperature (18° to 25°C).
- Combine 100 µl of each of the control serums (positive control and negative control) in the appropriate well (double determination is recommended, particularly for a large number of samples).
- For the null value, place 100 µl of sample dilution buffer in appropriate well (single determination).
- Place 100 µl of the pre-diluted sample in the appropriate wells. (single determination).
- Uncover the plate and incubate it for 60 minutes at room temperature (18° to 25°C).
- *First wash:* Empty the wells and wash them 4 times, each time with 300 µl of the wash buffer (1:10 dilution of the 10x wash buffer). If you are using a multi-channel pipette, wipe it on a clean and absorbent pad (e.g. paper towel) after each wash. If you are using an automatic washer, it is not necessary to wipe after each wash. After 4 rounds of washing, carefully remove any remaining liquids by wiping the plate on a clean and absorbent pad.
- In each well, put 100 µl of conjugate solution. Uncover the plate, and incubate it for 60 minutes at room temperature (18° to 25°C).
- *Second wash:* Empty all of the wells and repeat the procedure described above for the first wash.
- Put 100 µl substrate solution in each well. Uncover the plate and incubate it for 15 minutes at room temperature (18° to 25°C). Start timing the incubation as soon as the first well is filled.
- Put 100 µl stop solution in each well, in the same sequence and at the same speed that the substrate solution was put in the wells.
- Shake the plate carefully but thoroughly, or turn on the shake function of the plate photometer. Measure the extinction value (OD) at 450 nm within 10 minutes of introducing the stop solution.

EVALUATION OF THE RESULTS

1. Reference values to check correct test procedure

- Calculate the average of the optical density of the positive controls (PC) and negative controls (NC) (OD_{PC} , OD_{NC}).
- Calculate the percentage (P) of the optical density of the negative control serum according to the following formula:

$$P = \frac{OD_{NC} \times 100}{OD_{PC}}$$

- The test procedure was correct if the following reference values were obtained:

Positive control serum:	$OD_{PC} > 0.8 < 2.8$
Negative control serum:	$P < 20$
Null value (Sample dilution buffer):	$OD < 0.1$

- If the necessary values were not obtained, or if the substrate already showed a strong blue colouring before it was added to the wells, this can be an indication that the test was not correctly performed, that there was contamination, or that the reagents have expired. Before testing again, check the devices and materials that were used, check for contamination, and check the expiration dates of the reagents.

2. Calculating the Test Results

- Calculate the average of the optical density of the positive controls (PC) and negative controls (NC), (OD_{PC} , OD_{NC}).
- Subtract the average of the optical density of the negative controls from the average of optical density of the positive controls and from the optical density of the samples (OD_{Sample}).

$$OD_{PC, \text{ corr}} = OD_{PC} - OD_{NC}$$

$$OD_{Sample, \text{ corr}} = OD_{Sample} - OD_{NC}$$

- Calculate the Test Result (TR) according to the following formula:

$$TE = \frac{OD_{Sample, \text{ corr}} \times 100}{OD_{PC, \text{ corr}}}$$

3. Evaluating the Test Results

TE	< 8	negative
TE	8 - 11	inconclusive
TE	> 11	positive

INTERPRETATION OF THE TEST RESULTS

The interpretation of the test results and the consequences that follow should be decided by the attending veterinarian in a medical context, with careful attention to the case history, clinical symptoms, antibody titre dynamics (serodiagnostic follow-up tests), and diagnostically differentiated determination about other causes of illness.

STORAGE

All elements of the test kit should be stored at 2° to 8°C. Before use, bring the required number of microtitre stripes and the reagents to room temperature (18° to 25°C). Under no circumstances should the substrate solution be exposed to sunlight or other strong light sources! The elements of the test kit may not be mixed with the parts of other batches or other test kits.

PRECAUTIONS AND GENERAL WARNINGS

The usual prudence for ELISA tests — including the use of carefully cleaned containers, careful pipetting, and the sequential performance of individual test steps — is a necessary condition for achieving correct test results.











- Some elements of the test kit contain hazardous materials (sodium azide, ProClin 300). Disposal of waste should follow legally required procedures.
- This test apparatus is intended for *in-vitro* use, and may only be used by trained laboratory personnel strictly following the instructional information.
- Numerous components of the test apparatus may only be used prior to the expiration dates listed on the packaging.
- Mixing components from different batches is prohibited.
- Use of reagents from other manufacturers is prohibited.
- Some reagents contain trace amounts of sodium azide or ProClin 300 as a preservative. The concentrations of these hazardous materials is below the legal limit set forth in (EC) 1272/2008. Avoid contact with mucous membranes or ingestion.

- After the reagents have been removed, the packages should be re-sealed immediately and returned to their storage at 2° to 8°C. Please note that switching the caps, particularly the caps on the control serums can lead to cross-contamination of the test components, rendering them unusable.
- The test material should be considered potentially infectious. Legal requirements regarding prevention of accidents with potentially infectious material and with dangerous chemicals must be observed.
- In this context, the following additional rules and general directions should be followed:
 - Do not eat, drink or smoke!
 - Never operate the pipette with your mouth!
 - Always wear a lab coat and protective gloves!
 - Follow the safety warnings on every test component!
 - Carefully follow the instructions for use!
 - Safety measures and warnings should be read and followed!
 - The method of performing the test in full or half-automated form, e.g., with the use of automatic pipettes or lab robots, should be validated.
 - A combination of this test kit with products or components from other manufacturers is not permitted and can lead to false test results.

LIABILITY

All liability in connection with the use of this product is assumed by the purchaser. The manufacturer assumes no liability for damages of any sort that result from the use of this test kit, from performing the test, or from the evaluation and interpretation of the test results produced by the product.

For veterinary use only.

	Order number		Expiration date
	Lot number		Storage temperature
	Manufacturer		<i>In vitro</i> diagnostic
	Follow instructions		Do not expose to sunlight
	Single-use		Do not expose to moisture