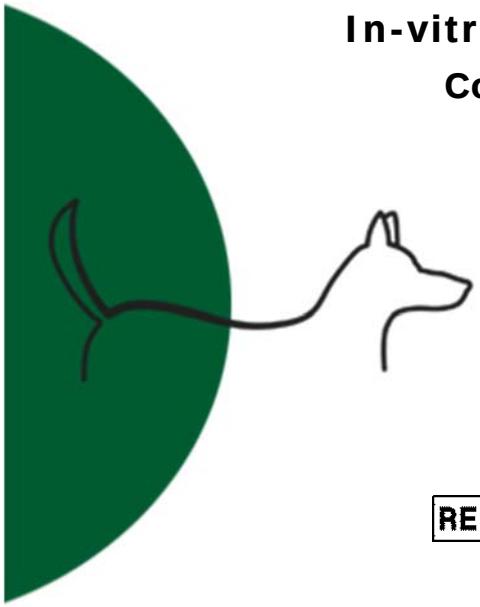
Animal Welfare & Food Safety GmbH afosa Gesunde Tiere = Sichere Lebensmittel

Instructions for use

LEISHMANIA-ELISA DOG

Test to detect the presence of antibodies against the cause of leishmaniosis, *Leishmania infantum*

In-vitro Diagnostic for Dogs



Contents of the Kit:

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

REF LED-Kit

LOT Lot number: see sticker

Expiration date: see sticker

Reg.-No.: FLI-B 498

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IVD

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

PRODUCT DESCRIPTION

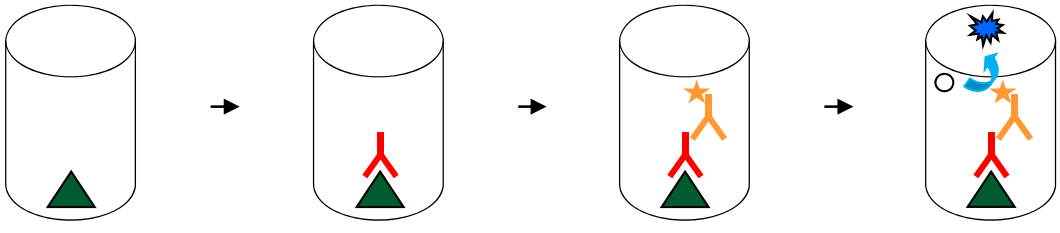
LEISHMANIA-ELISA DOG is an *in-vitro* diagnostic test to detect the presence of IgG antibodies against *Leishmania infantum*, the pathogen of the leishmaniosis, in samples of dog serum.

GENERAL INFORMATION

The leishmaniosis is a zoonosis which spreads across the tropics, subtropics, southern Europe as well as the Mediterranean area through to the 45th latitude at which dogs as reservoir hosts are infected at a rate of < 1% up to 37%. The leishmaniosis of dog (causative organism is *Leishmania infantum*) has even a noteworthy meaning in far northern areas as a travel or an imported disease. The pathogens are transmitted by sandflies (Phlebotomidae). Due to an incubation period ranged from months to years dogs of all ages can be infected. The disease can proceed symptomatically (accompanied by polyclonal B-cell-activation; generation of specific antibodies and cellular immunosuppression), subclinically (accompanied by specific humoral immune response) or latently (lack of respectively very weak humoral and protective cellular immune response). Clinical symptoms are (ordered by decreasing frequency) lymphadenopathy, skin reactions, cachexia, hyperthermia, conjunctivitis, splenomegalia and abnormal claws. Often slight to moderately normochromic anemia, moderate leukopenia, hypergammaglobulinemia and hypoalbuminemia are observed. The suspected diagnosis is based on clinical, haematological and anamnestic information and can be confirmed in most cases - except of latent infections without humoral immune response - by detection of specific anti *leishmania* antibodies using this ELISA.

DESCRIPTION OF THE TEST

The testing system detects the presence of IgG antibodies against *Leishmania infantum* in samples of dog serum. The microtitre plates are coated with a preparation of *Leishmania* antigens. During the incubation period of the serum sample, antibodies against *Leishmania infantum* 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-*Leishmania* antibodies present in the sample. Diagnostic evaluation is made by comparing the extinction values of samples against controls.



Test plate with *Leishmania* antigen

Sample with anti-*Leishmania*-IgG antibody

Conjugate with enzyme-marked anti-dog IgG antibody

Enzyme-induced colour change in the substrate

TEST KIT CONTENTS

- The reagents in one test kit are sufficient for 91 evaluations.

- Microtitre plate (1 plate):
  Coated with *Leishmania*-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, 1.5 ml): **RED cap**
  Serum of dogs infected with *Leishmania infantum*, preserved with sodium azide, ready-to-use

- Negative control serum PC (1 bottle, 1.5 ml): **GREEN cap**
  Serum of dogs not infected with *Leishmania infantum*, preserved with sodium azide, ready-to-use

- Conjugate solution (1 bottle, 12 ml): **RED cap**
  Horseradish peroxidase-conjugated anti-dog IgG immunglobulins, 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 10 µl of the sample with 90 µl of the sample dilution buffer (1:10).
Mix the thinned solution thoroughly. Afterwards add 10 µl of the 1:10 mixture in 290 µl of sample dilution buffer, so that an end-dilution of 1:300 of the blood serum is made.

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, of 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}).

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

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

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

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

3. Evaluating the Test Results

TE	< 7	negative
TE	7 - 12	inconclusive
TE	> 12	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 mictrotitre 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 appartus 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		*In vitro* diagnostic
	Follow instructions		Do not expose to sun-light
	Single-use		Do not expose to mois-ture