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Canine Babesia Antibody (Babesia-Ab)ELISA Kit

User's Manual

Catalogue Number: SL0301Ca

48 or 96 Tests

For Research Use Only

Store all reagents between 2-8℃

Validity Period: Six (6) months

For use with serum, plasma, culture media, or any biological fluids.

Read the Materials Safety Data Sheet (MSDS) and follow all handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Materials Safety Data Sheets (MSDS) are available at https://www.sunlongbiotech.com/article.php?id=1762

FOR RESEARCH USE ONLY.

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS

PLEASE FOLLOW THE KIT INSTRUCTIONS TO PERFORM THE PROCEDURE.

Canine Babesia Antibody (Babesia-Ab) ELISA Kit

FOR RESEARCH USE ONLY

Product Information

Intended Use

Enzyme immunoassay for the qualitative detection of Canine Babesia-Ab in serum, plasma, culture media, or any biological fluid.

Principle

The qualitative detection of Canine Babesia-Ab is based on the ELISA (Enzyme-Linked Immunosorbent Assay) technique. The microplate wells are pre-coated with an antigen specific to Canine Babesia-Ab. The corresponding antibody in the sample or standards binds the immobilized antigen after incubation. After washing the microplate wells to remove all unbound sample material, horseradish peroxidase (HRP) labeled antigen conjugate is added, which binds to the antibody, forming antigen-antibody-enzyme-labeled antigen complex upon incubation. In a second wash step, all unbound material is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue color reaction in wells containing the complex. A stop solution containing acid terminates the reaction, producing a yellow end-point coloration proportional to the amount of specific antibody present in the sample. Absorbance is measured at 450 nm using an ELISA microwell plate reader. Analysis is completed by comparing the OD values of samples to a cut-off value.

Reagent and Materials provided with the kit

	Materials provided with the kit	96T	48T	Storage
1	Instructions for Use	1	1	R.T.
2	Adhesive films/membrane	2	2	R.T.
3	Sealed bags	1	1	R.T.
4	Microplate	1	1	2-8℃
5	Negative Control	0.5ml×1 bottle	0.5ml×1 bottle	2-8℃

6	Positive Control	0.5ml×1 bottle	0.5ml×1 bottle	2-8℃
7	HRP-Conjugate Reagent	6ml×1 bottle	3ml×1 bottle	2-8℃
8	Sample Diluent	6ml×1 bottle	3ml×1 bottle	2-8℃
9	Chromogen Solution A	6ml×1 bottle	3ml×1 bottle	2-8℃
10	Chromogen Solution B	6ml×1 bottle	3ml×1 bottle	2-8℃
11	Stop Solution	6ml×1 bottle	3ml×1 bottle	2-8℃
12	Wash Solution Concentrate	20ml (30X*) ×1bottle	20ml (20X*) ×1bottle	2-8℃

*X denotes the product is concentrated 30 or 20 times for 96 and 48 tests, respectively

For potential hazardous substances please check the safety data sheet.

The kit allows the performance of 48 or 96 reactions, including blank, negative, and positive controls.

Materials required but not provided

1. ELISA Microtiter plate reader, equipped for the measurement of absorbance at 450 nm
2. Incubator at 37℃
3. Manual or automatic equipment for rinsing Microtiter plates
4. Pipettes to deliver volumes between 10 and 1000 µl
5. Vortex tube mixer
6. Distilled/Deionized water
7. Graduated measuring cylinder
8. Wash bottle
9. Paper towels/absorbent paper
10. Timer
11. Disposable tubes

Storage

Store the kit at 2 - 8°C. The opened reagents are stable up to the expiry date stated on the kit when stored at 2 - 8°C. Return the unused plate strips to the aluminum foil bag immediately, squeeze out air and seal tightly. Keep the desiccant inside and store at 2-8℃ refrigerated. Use up within 1 month, max 3 months. Discard directly if mold, condensation or uneven liquid adhesion on well walls is found.

Sample preparation

1. **Serum**

Collect whole blood into a non-anticoagulated (plain, red-top) tube. Allow the blood to clot undisturbed at room temperature for 10–20 minutes. Then centrifuge at 2,000–3,000 rpm for 20 minutes to separate the serum by removing the clot. If precipitates form during storage, centrifuge the sample again before use

2. **Plasma**

Collect whole blood into tubes containing anticoagulants such as EDTA or citrate. After incubating at room temperature for 10–20 minutes, centrifuge at 2,000–3,000 rpm for 20 minutes. Carefully collect the plasma supernatant. If precipitates form during storage, centrifuge the sample again before use.

3. **Urine**

Collect urine in sterile tubes. Centrifuge at 2,000–3,000 rpm for 20 minutes and carefully collect the clear supernatant. If precipitates appear during storage, centrifuge the sample again. Prepare **cerebrospinal and pleuroperitoneal fluids** following the same procedure as for urine.

4. **Cell samples**

Collect culture supernatant aseptically and centrifuge at 2,000–3,000 rpm for 20 minutes. Carefully collect the clarified supernatant. To detect intracellular components, dilute cells to 1×10^6/mL with Phosphate Buffered Saline (PBS) pH 7.2–7.4 and lyse by repeated freezing and thawing. Centrifuge the lysate under the same conditions and collect the supernatant. If precipitates appear during storage, centrifuge again before use.

5. **Tissue samples**

Tissue samples are cut, weighed, frozen in liquid nitrogen, and stored at -80℃ until use. Homogenize the tissue in PBS (pH 7.4) on ice or at 4℃. Centrifuge at 2,000–3,000 rpm for 20 minutes and carefully collect the supernatant. Aliquot the supernatant for ELISA and future analysis.

Note that for processing methods for uncommon ELISA samples, visit https://www.sunlongbiotech.com/article.php?id=1735

Notes:

1. Perform sample preparation and ELISA assay promptly after collection. Extract samples following relevant protocols. If immediate testing is not possible, store samples at -20°C. Avoid repeated freeze-thaw cycles to preserve sample integrity

2. Our kits are not compatible with samples containing Sodium Azide (NaN_3), as it inhibits the activity of horseradish peroxidase (HRP), compromising assay performance.

3. It is very important to bring all reagents and samples to room temperature and mix them before starting the procedure

Preparation of reagents

Wash Solution Concentrate should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the Wash Solution Concentrate, warm at 30 – 40°C until they have completely dissolved.

Wash Solution (1X)

1. Pour the entire contents (20 mL) of the Wash Solution Concentrate (20X for 48 tests or 30X for 96 tests) into a clean 1,000 mL graduated cylinder. Bring to final volume to 400mL for 48 test or 600 mL for 96 tests with distilled or deionized water.
2. Mix gently to avoid foaming.
3. Transfer to a clean wash bottle and store at 2–25°C.

Please note that Wash Solution (1X) is stable for 30 days.

Procedure

1. **Preparation of Microplate.** Label the microplate wells sequentially with the corresponding sample numbers. Include two wells, each for positive and negative controls, assayed in duplicate, and one empty well as a blank control (well A1). Do not introduce samples or HRP Conjugate reagent into the blank well (well A1); however, continue to perform all subsequent steps as per the standard protocol. Remove any unused microplate strips from the holder and store them sealed in the provided foil bag with desiccant at 2–8°C.
2. **Pipetting the controls and samples.** Add 50 µL of negative and positive controls to their respective wells. For sample wells, add 40 µL of sample diluent followed by 10 µL of sample. Carefully dispense samples to the bottom of the wells without touching the walls. Mix gently by shaking to ensure proper mixing.
3. **Incubation.** Incubate the Microplate 30 minutes at 37°C after sealing with an Adhesive film/membrane.
4. **Plate Washing.** Wash the strips five (5) times with 400 µL of 1X Wash Solution per well.

Soak strips for 30 seconds in between washes. When using an automated plate washer, be sure to recalibrate it so that the tips do not touch the bottom of the wells. Also, take caution when manually dispensing and aspirating into and out of the wells/ Remove residual Wash Buffer by tapping the plate upside down on paper towels without letting the wells dry out.

Note: Washing is important! Insufficient washing results in poor precision and false results.

5. **Conjugate Addition.** Add 50 µL HRP-Conjugate reagent to each well except the blank control well (well A1).

6. **Conjugate Incubation.** Incubate the Microplate 30 minutes at 37°C after sealing with an Adhesive film/membrane as in Step 3.

7. **Plate Washing.** Wash the strips five (5) times with 400 µL of 1X Wash Solution per well. Soak strips for 30 seconds in between washes. Remove residual Wash Buffer by tapping the plate upside down on paper towels without letting the wells dry out, as in Step 5. Do not touch the bottom of the wells either during manual or automated washing.

Note: Washing is important! Insufficient washing results in poor precision and false results.

8. **Substrate Addition and Incubation.** Add 50 µL Chromogen Solution A and 50 µL Chromogen Solution B to each well, mix with gentle shaking, and incubate at 37°C for 15 minutes. This should be done while avoiding a light source.

9. **Stop Reaction**. Add 50 µL of Stop Solution to each well to terminate the enzymatic reaction. Upon addition, the color will change from blue to yellow, indicating the reaction has been successfully stopped

10. **Measurement.** Remove any drops of water and fingerprints on the bottom of the plate and confirm there are no bubbles on the surface of the liquid. Measure the optical density (O.D.) at 450 nm using a microtiter plate reader. Set the O.D. value of the blank control well to zero. Complete all readings within 15 minutes after adding the stop solution to ensure accurate results.

Note that due to technical reasons, if the ELISA Microtiter plate reader cannot be adjusted to zero using the Blank, subtract the Blank absorbance value from all other absorbance values measured to obtain reliable results

Evaluation of the result

Test results are accounted as valid if the

1.average value of positive control ≥1.00.

2.average value of negative control ≤0.10.

The critical value (Cut Off) calculation:

Critical value = average value of negative control + 0.15

Negative Result:

OD value < Cut Off, the sample is Canine Babesia-Ab negative.

Positive Result:

OD value ≥ Cut Off, the sample is Canine Babesia-Ab positive.

Precautions

1. Store the kit at 2-8°C upon receipt. Before use, bring the kit to room temperature. Remove any unused strips from the Canine PIV antigen-coated plate, reseal them in the provided zip-lock foil bag with desiccant, and keep at 2-8°C.

2. Precipitates may form in the concentrated wash buffer. Warm the buffer to dissolve precipitates; this will not affect assay performance.

3. To prevent cross-contamination, use adhesive film/membrane only once.

4. Protect the substrate from exposure to light to maintain its stability and reactivity.

5. Follow the manufacturer's instructions carefully. Results must be read using a microplate reader.

6. Treat all samples, wash buffer, and waste as potentially infectious.

7. Do not mix reagent from different kit lots.

Storage and Validity

1. Storage: 2-8℃.

2. Valid Duration: 6 months

Troubleshooting

Possible Case	Solution
Poor Standard Curve	
• Improper standard curve preparation	• Ensure accurate operation of the dilution
• Incomplete washing and aspiration	• Adequate washing and adequate aspiration
• Inaccurate Pipetting	• Check and Calibrate pipettes

High Background	
• Improper washing	• Increase the duration of soaking before aspiration
• Contaminated Substrate	• Replace Substrate. Use a clean substrate and avoid cross-contamination by replacing the Adhesive film/membrane
• Non-specific binding of the antibody	• Replace with another purified antibody or blocking buffer
• The plate is not sealed completely	Follow the instructions strictly
• Incorrect incubation temperature	• Incubate at 37℃
• Substrate exposed to light before use	• Keep the substrate in a dark place
• Contaminated wash buffer	• Use a clean buffer and a sterile filter

Weak Signal	
• Improper washing	• Increase the duration of soaking before aspiration
• Incorrect incubation temperature	• Incubate at 37℃
• Not enough antibody were used	• Increase the concentration of the antibody
• Reagents are contaminated	• Use new reagents
• Pipettes are not clean	• A clean pipette should be used

No Signal	
• Reagents are contaminated • Sample prepared incorrectly	• Use new reagents • Make sure samples are diluted accordingly
• Not enough antibody were used • Wash buffer contains sodium azide • HRP was not added	• Increase the concentration of antibody • Use a new wash buffer without sodium azide • Add HRP according to the instructions
Poor Precision	
• Imprecise/ inaccurate pipetting • Incomplete washing of the wells	• Check and calibrate pipettes • Ensure wells are thoroughly washed by filling each well with wash buffer and completely removing all residual antibody solutions before proceeding to the next wash step