

BRUCELLA cELISA

BRUCELLA S ANTIBODY TEST KIT, cELISA

The BRUCELLA S ANTIBODY TEST KIT cELISA is a qualitative test that uses competitive ELISA technology. The test determines the presence of antibodies against Brucella species, which produce smooth colonies (*B. melitensis, B. abortus* and *B. suis* - Rev. sci. tech., OIE 1982) in **individual serum and plasma samples**. The presence of target antibodies indicates a prior infection with smooth Brucella biovars. Recommended use for the product is for screening or confirmatory testing.

Brucella cELISA has been validated in **bovine serum/plasma, sheep and goat serum samples.**

The diagnostic test uses microtiter plates containing wells coated with lipopolysaccharide (LPS) extracted from *Brucella abortus* bacteria. Any anti-Brucella antibodies present in the sample will compete with LPS specific, mouse anti-Brucella monoclonal antibodies on the LPS specific binding site. In case of presence of Brucella antibodies in tested samples, mouse anti-Brucella monoclonal antibodies will be blocked from binding. After a wash step, HRP-conjugated secondary anti-mouse antibodies (Conjugate) are added, and will bind to any immobilized mouse anti-Brucella monoclonal antibodies in the wells. Following another wash step, any bound Conjugate is revealed using a TMB substrate that produces color in the presence of HRP. A microplate reader is used to measure the optical density of the color produced. The amount of color generated is inversely proportional to the amount of specific anti-Brucella antibodies in the animal serum.

Kit Contents

Reagents	2-plate Kit	5-plate Kit
Positive Control	**2 ml**	**4 ml**
Ready-to-use; bovine positive serum against *Brucella abortus*. Contains 0.095% sodium azide as a preservative. Hazard Code: Not classified according to EU regulations.		
Negative Control	**2 ml**	**4 ml**
Ready-to-use; bovine negative serum against *Brucella spp.* Contains 0.095% sodium azide as a preservative. Hazard Code: Not classified according to EU regulations.		
Antibody Diluent	**30 ml**	**50 ml**
Ready-to-use; proprietary formulation containing non-hazardous substances dissolved in ultrapure water. Hazard Code: Not classified according to EU regulations.		
100X Conjugate	**0.5 ml**	**1 ml**
A proprietary formulation containing purified animal antibodies. Hazard Code: Not classified according to EU regulations.		
20X Antibody Concentrate	**0.7 ml**	**1.7 ml**
A proprietary formulation containing purified animal antibodies. Hazard Code: Not classified according to EU regulations.		
Substrate	**30 ml**	**70 ml**
Ready-to-use, TMB-buffered solution. Hazard Code: Not classified according to EU regulations.		
10X Wash Buffer	**100 ml**	**2 x 100 ml**
A proprietary formulation containing non-hazardous substances dissolved in ultrapure water.. Hazard Code: Not classified according to EU regulations.		
Conjugate Diluent	**30 ml**	**70 ml**
A proprietary formulation containing animal serum albumin. Hazard Code: Not classified according to EU regulations.		
Stop Solution	**30 ml**	**70 ml**
Ready-to-use; low concentration acid solution. Hazard Code: **R35** - Causes severe burns; **S26** - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; **S36/37/39** - Wear suitable protective clothing, gloves, and eye/face protection; **S45** - In case of an accident or if feeling unwell, seek medical advice immediately (show the label on the vial).		
Brucella LPS Coated Plates	**2 plates**	**5 plates**

Brucella abortus LPS coated on microplates.
Hazard Code: Not classified according to EU regulations.

Materials Required But Not Provided

- Precision single and multi-dispensing micropipettes and tips for volumes between 10 to 1000 µl (*e.g.* single pipettes 10-100 and 100-1000 µl and multichannel pipette 50-300 µl)
- Test tubes or non-antigen-coated transfer plate(s) with flat or "U" bottom for sample diluting
- Plastic or glass bottles with screw caps, laboratory beakers or Erlenmeyer flasks to make ready-to-use Wash Buffer
- Reagent reservoirs to transfer reagents into plates
- ELISA microplate reader or spectrophotometer equipped with 450 nm filter
- Deion-ized, distilled, or RO purified water to make up the Wash Buffer
- Manual or automatic microplate washing system
- Incubator capable of maintaining a temperature of +37°C
- Microplate cover lids or adhesive foil to cover plates
- Microplate shaker and vortex mixer

For supplies contact our customer support at support@ellielab.com.

Storage & Stability

The 20X Antibody Concentrate and the 100X Conjugate must be stored at -20± 5˚C. All other reagents must be stored at 2-8°C.

Do not equilibrate 20X Antibody Concentrate and 100X Conjugate to room temperature for use. Use directly from the freezer to make ready to use components. Return to the freezer after use. All other reagents of the kit must be equilibrated to room temperature (20-25°C) for up to 90 minutes before use.

Kit is transported in a cooled box at a temperature be-tween 0 and 15°C.

Do not use components after the expiration date. Do not mix reagents from different kit lots. Do not expose TMB solution to strong light or oxidizing agents. Handle TMB solution with clean glass or plastic ware.

Care should be taken to prevent contamination of kit components.

Warnings

- All reagents are for *in vitro* diagnostic use only.
- Do not pipette by mouth.
- Avoid contact with open skin.
- Sodium azide is a toxic substance and is used in some reagents. In case of contact with eyes or skin, flush immediately with copious amounts of water. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of reagents, flush with a large volume of water to help prevent azide build-up.
- The Stop Solution contains dilute acid. Use with care to avoid contact with skin and eyes. Avoid exposure to bases, metals, or other compounds that may react with acids. Spills should be cleaned up immediately.

All materials in this kit should be treated according to the product Safety Data Sheet.

Specimen Requirements

Ellie's Brucella cELISA test can be performed with serum/plasma samples from cattle and serum samples from sheep and goats.

The test uses only 100 µl of sample per duplicate test. Collect the amount of blood required by the blood collection system.

Collect blood aseptically in untreated tubes, serum separator tubes or Li-Heparin tubes. Allow blood to clot and separate serum. Centrifuge blood collected with Li-Heparin tubes at a minimum 4500 RPM for 5 minutes and separate the plasma. Avoid the use of heavily hemolyzed or contaminated samples. Store samples at 2-8˚C. Freeze samples at -20˚C if not tested within 72 hours; avoid repeated freezing.

Preliminary Steps

Reagent Preparation

All reagents of the kit must be equilibrated to room temperature (20-25˚C) before use, except for **20X Antibody Concentrate and 100X Conjugate**. The minimum time needed to do this is 90 minutes.

Remove the antigen-coated plate from the foil pouch. If using partial plates, only remove the number of wells needed to test all samples. Place the remaining wells back into the pouch and return them to 2-8˚C.

Wash Buffer Preparation

Prepare ready-to-use Wash Buffer by mixing one part 10X Wash Buffer with 9 parts distilled or deionized water. It is very important to equilibrate the Wash Buffer to room temperature before use. Mix well. The amount of Wash Buffer needed to wash one plate is 300 ml. Store the Wash Buffer at room temperature up to one month.

Competing Antibody Preparation

Use the 20X Antibody directly from the freezer to make ready to use solution. Mix the concentrated 20X Antibody Concentrate gently before making the ready-to-use solution. Dilute concentrated 20X Antibody Concentrate 1:20 with Antibody Diluent by combining one part 20X Antibody Concentrate with 19 parts Sample Diluent (*e.g.* the quantity needed for one plate is prepared by mixing 260 µl of concentrated Antibody and 4.94 ml of Antibody Diluent). **Return the 20X Antibody Concentrate to -20±5˚C after use**. The prepared working dilution of Antibody must be used the same day it is prepared!!

Conjugate Preparation

Use the 100X Conjugate directly from the freezer to make ready to use solution. Mix the concentrated 100X Conjugate before making the ready-to-use solution. Mix the concentrated 100X Conjugate 1:100 with Conjugate Diluent by combining one part 100X Conjugate with 99 parts Conjugate Diluent (e.g. the quantity needed for one plate is prepared by mixing 110 µl concentrated Conjugate and 10.89 ml Conjugate Diluent). **Return the 100X Conjugate to -20±5˚C after use.** Protect the prepared dilution from light. The prepared working dilution of Conjugate must be used the same day it is prepared!

Preparation of samples and controls

Pre-dilute controls and samples in 1:1 ratio with Antibody (*e.g.* 60 µl of controls or samples with 60 µl of Antibody). Pipette Controls in duplicate. Use transfer plates or microtubes. Mix well before further processing. For microplates, use a plate shaker; for tubes, use a vortex mixer, if available.

Testing procedure

1. Pipette 100 µl of prediluted controls and samples into Brucella LPS Coated Plate test wells.
2. Cover the plate and incubate it for one hour at 37 ℃ or at room temperature overnight.
3. Wash the plate:
    - Discard the plate contents.
    - Wash four times with 300 ± 20 µl of Wash Buffer.
    - Tap the plate firmly on ab-sorbent paper after the last wash step.
4. Dispense 100 µl of Conjugate into each well.
5. Incubate for 30 minutes at room temperature. Do not cover the plate.
6. Wash the plate:
    - Discard the plate contents.
    - Wash four times with 300 ± 20 µl of Wash Buffer.
    - Tap the plate firmly on ab-sorbent paper after the last wash step.
7. Dispense 100 µl of Substrate into each well.
8. Incubate for 15±3 minutes at room temperature.
9. Add 100 µl of Stop Solution into each well.
10. Read the test results at 450 nm on a microplate reader.

Test Validation

- The mean O.D. of the Negative Controls must be > 0.800.
- The mean O.D. of the Positive Control must be < 0.400.

If validation criteria are not met, test results are invalid and the samples must be retested.

Results & Interpretation

Calculate Percent of inhibition

PI=100 - ((OD Sample x 100)/OD NC)

OD Sample = OD value of the sample or the Positive Control

OD NC = Mean OD value of the Negative Control

Interpretation

Results are interpreted according to the following tables:

Cutoff values for EU countries and non vaccinated animals:

Negative	Positive
≤ 35	> 35

Cutoff values for the rest of the world and vaccinated animals:

Negative	Positive
≤ 50	> 50

Positive samples must be retested in duplicate. If any of the retests read > 50, the sample is considered positive.

Cutoff values may vary from country to country, for different use or vaccination status of animals.

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