

BRUCELLA LPS iELISA

BRUCELLA S ANTIBODY TEST KIT, LPS iELISA

The BRUCELLA S ANTIBODY TEST KIT, LPS iELISA is a semi-quantitative test that uses indirect ELISA technology. The test determines the presence of antibodies against Brucella species that produce smooth colonies (*B. melitensis, B. abortus*, and *B. suis* - Rev. Sci. Tech., OIE 1982) in individual serum/plasma or pooled bovine serum samples (pools of maximum 5 samples - EU Council Directive 64/432/EEC, Annex C), individual sheep/goat serum samples and individual milk samples from cattle. The presence of antibodies indicates a recent Brucella infection.

The diagnostic test uses microtiter plates containing wells coated with lipo-polysachharide (LPS) extracted from *Brucella abortus* bacteria. Any anti-Brucella antibodies present in the sample will bind to the LPS coated on the plate. After a subsequent wash step, secondary HRP-conjugated antibodies (Conjugate) will bind to any immobilized antibodies in the wells. Following another wash step, any bound Conjugate is detected 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 proportional to the amount of specific anti-Brucella antibodies in the animal serum or milk sample.

Kit Contents

Reagents	2-Plate Kit	5-Plate Kit
Positive Control	**0.5 ml**	**1 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	**0.5 ml**	**1 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.		
Sample Diluent	**60 ml**	**120 ml**
Ready-to-use; proprietary formula containing animal serum. Sample Diluent contains 0.1% ProClin 300 as a preservative. 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.		
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 formula containing 0.1% ProClin 300. 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.		
Brucella LPS Coated Plate	**2 plates**	**5 plates**
Brucella abortus LPS coated on microplates. 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).		

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 multi-channel pipettes 5-50 and 50-300 µl)
- Test tubes or non-antigen-coated transfer plate(s) for sample diluting
- Plastic or glass bottles with screw caps and, laboratory beakers or Erlenmeyer flasks to make ready-to-use Wash Buffer
- Reagent reservoirs to transfer reagents onto plates
- ELISA microplate reader or spectrophotometer equipped with a 450 nm filter
- Deion-ized or distilled 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 kit must be stored at 2-8˚C. The kit is transported in a cooled box at a temperature between 0 and 15°C.

Do not use components after the expiration date. Do not mix reagents from different kit lots. Do not expose the 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 it 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 and 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 LPS iELISA test can be performed with serum or plasma samples from cattle, serum samples from sheep and goats and milk samples from cattle.

Serum and Plasma Samples

The test uses only 20 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.

Milk Samples

The test uses only 100 µl of milk per duplicate test. Do not use colostrum or milk samples up to 20 days after calving for testing individual milk samples because of nonspecific reactions. Individual milk samples should be collected before the milking process. Collect the milk samples from the healthy quarter of the udder by throwing away the first 5-10 jets of milk and then collect the sample.

Milk samples should be transported in a cooled box at a temperature be-tween 0 and 15°C. For short-term storage up to 3 days, keep the milk samples at 2-8 °C. For long-term storage, keep the milk samples at -20℃.

Preliminary Steps

Reagent Preparation

All kit reagents must be equilibrated to room temperature (20-25˚C) before use, except for 100X Conjugate. The minimum time needed to do this is 2 hours. It is very important to heat the Sample Diluent and Wash Buffer to room temperature before use.

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.

Conjugate Preparation

Dilute 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 of concentrated Conjugate and 10.89 ml of Conjugate Diluent) and mix well. Return the 100X Conjugate to 2-8˚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

Mix samples well before testing. Hemolyzed samples are acceptable for testing. Lyophilized samples should be reconstituted completely, and frozen samples should be fully thawed and mixed.

Pre-dilute controls, and individual serum/plasma or pooled serum samples (composed of maximum 5 serum samples) 1:10 in Sample Diluent (*e.g.,* 10 µl of controls or samples in 90 µl of Sample Diluent). Use transfer plates or microtubes. Mix well before further processing. For microplates, use a plate shaker; for tubes, use a vortex mixer, if available. **Milk samples should be tested undiluted.**

Testing procedure

1. Load samples into a Brucella LPS Coated Plate.

   Controls, serum and plasma samples:

   Pipette 90 µl of Sample Diluent into all Brucella LPS Coated Plate test wells. Immediately add 10 µl of pre-diluted samples and controls into their respective wells. Pipette controls in duplicate for each plate.

   Individual milk samples:

   Pipette 50 µl of the Sample Diluent into all Brucella LPS Coated Plate test wells. Add 50 µl of each undiluted milk sample.

   NOTE: If there is a large number of individual milk samples to be tested, transfer the samples to a transfer plate first. Mix each milk sample thoroughly by hand right before adding it to its respective well on the transfer plate. Use a multichannel pipette to pipette the milk samples into the Brucella LPS Coated Plate test wells. Mix the milk samples by pipetting them with multichannel pipette tips 1-3 times and immediately pipette the appropriate amount into the test wells.

   If the milk samples are added directly to test wells of the Brucella LPS Coated Plate, mix the samples thoroughly by hand right before adding them to test wells.

2. Mix the test plate well before further processing. Use a plate shaker if available.
3. Incubate it for 30-40 minutes at room temperature.
4. 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.

5. Dispense 100 µl of Conjugate into each well.

6. Incubate it for 30-40 minutes at room temperature.
7. 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.

8. Dispense 100 µl of Substrate into each well.
9. Incubate for 15 ± 3 minutes at room tempera-ture.
10. Dispense 100 µl of Stop Solution into each well.
11. Read the results at 450 nm on a microplate reader.

Test Validation

- The mean OD of the Negative Controls must read less than 0.200 OD.
- The mean OD of the Positive Controls must read over 0.800 OD.

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

Results & Interpretation

Calculation of Sample to Positive Ratio

$$\text{S/P Ratio} = \frac{\text{OD Sample - OD NC}}{\text{OD PC - OD NC}} \times 100$$

where:

OD Sample = OD value of a sample

OD NC = Mean OD value of the Negative Control

OD PC = Mean OD value of the Positive Control

Interpretation

Results are in-terpreted according to the following tables:

Individual serum, plasma samples and individual milk samples

Negative	Positive
≤ 25	> 25

Pooled serum samples

Negative	Positive
< 5	≥ 5

At this cutoff, a less than 0.5% false-positive rate is expected. For more aggressive campaigns, a lower cutoff is recommended with an expected false positive rate of less than 1%. Call us for consultation.

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