

BRUCELLA OPS iELISA Sheep & Goats

BRUCELLA ANTIBODY TEST KIT, OPS iELISA Sheep & Goats

The BRUCELLA ANTIBODY TEST KIT, OPS iELISA Sheep & Goats is a semi-quantitative test that uses indirect ELISA technology. The test determines the presence and titer of antibodies against O-Chain polysaccharide (O-polysaccharide or OPS) of *Brucella spp.* in individual sheep and goat serum samples. The presence of antibodies against OPS indicates a recent or current infection with smooth Brucella biovars. *Brucella melitensis* and *Brucella abortus* share enough OPS epitopes to enable high sensitivity and specificity when *B. melitensis* is the infecting biovar.

The diagnostic test uses microtiter plates containing wells coated with OPS fragments of various lengths extracted from *Brucella abortus* bacteria. Any anti-*Brucella* OPS antibodies present in the sample will bind to the OPS 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 OPS antibodies in the animal serum sample.

Kit Contents

Reagents	2-Plate Kit	5-Plate Kit
Positive Control	**0.5 ml**	**1 ml**
Ready-to-use; sheep or goat positive serum against *Brucella spp.* 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; sheep 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 OPS Coated Plate	**2 plates**	**5 plates**
Brucella abortus OPS 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 OPS iELISA Sheep & Goats test can be performed with serum samples from sheep and goats.

Serum 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 or serum separator tubes. Allow blood to clot and separate serum. 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 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 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). 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.

Pre-dilute controls and 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.

Testing procedure

1. Load pre-diluted samples and controls into a Brucella OPS Coated Plate.
   - Pipette 90 µl of Sample Diluent into all Brucella OPS 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.
2. Mix the test plate well before further processing. Use a plate shaker if available.
3. Cover the plate and incubate it for 60 minutes at 36-38°C.
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. Cover the plate and incubate it for 60 minutes at 36-38°C.
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 interpreted according to the following table:

Individual serum samples

Negative	Positive
< 30	≥30

ellie

N114 W19320 Clinton Dr., Unit 5
Germantown, WI 53022, United States of America
Phone: +1 (800) 556-6953
support@ellielab.com