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BRUCELLA FPA

BRUCELLA ABORTUS ANTIBODY TEST KIT, FPA

The BRUCELLA ABORTUS ANTIBODY TEST KIT, FPA is a semi-quantitative test that uses Fluorescence Polarization technology to determine the presence of antibodies in serum, plasma, or milk samples against Brucella species that produce smooth colonies (*B. melitensis*, *B. abortus*, and *B. suis* - Rev. Sci. Tech., OIE 1982). The presence of antibodies indicates a current or recent Brucella infection.

The Brucella FPA has been validated for testing samples in bovine (bison, buffalo, and cervids). The kit can test serum and plasma samples. With the addition of ClearMilk™ Buffer, it is suitable for processing individual milk samples. Contact us for more details.

The diagnostic test uses an O-polysaccharide (OPS) extracted from *Brucella abortus* bacteria and conjugated with fluorescein. A fluorescence polarization instrument measures the polarization state of the light emitted by the OPS conjugate (Tracer). When no antibodies are present, the polarization is low. Polarization increases when antibodies bind to the Tracer.

Kit Contents

Reagents	250 tests	1000 tests
Positive Control	**1 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	**2 ml**	**2 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.		
25X Sample Diluent	**50 ml**	**50 ml**
Proprietary formula; Sample Diluent is a mixture of non-hazardous substances dissolved in ultrapure water. Hazard Code: Not classified according to EU regulations.		
Tracer	**2.5 ml**	**10 ml**
Ready-to-use; proprietary formula that contains O-polysaccharide (OPS) extracted from *Brucella abortus* bacteria labeled with fluorescein. Contains 0.095% sodium azide as a preservative. Hazard Code: Not classified according to EU regulations.		

Materials Required But Not Provided

- An FP instrument
- 10 x 75 or 12 x 75 mm borosilicate glass test tubes for tube instruments
- Sentry Microplate Strips for strip readers
- Black microtiter plates for microplate instrument
- Precision single micropipettes and tips for volumes between 10 to 1000 µl
- Plastic or glass bottles with screw caps and laboratory beakers or Erlenmeyer flasks to make ready-to-use Sample Diluent
- Distilled or deionized water
- ClearMilk™ Buffer for testing milk samples (Cat# C1001)

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

Storage & Stability

The kit should be stored at 2-8 ℃. The 25X Sample Diluent can be stored at room temperature.

During use, avoid exposing the Negative Control to temperatures higher than room temperature (up to 25°C).

The kit is transported in a cooled box at temperatures between 0 and 15°C.

Warnings

- All reagents are for *in vitro* diagnostic use only.
- Do not pipette by mouth.
- Avoid contact with open skin.
- Avoid pipetting that creates bubbles.
- Polarization readings are affected by temperature; all reagents used in the test should be at the same temperature as the samples being tested. Avoid temperature variations during testing.
- 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 disposing of reagents, flush with a large volume of water to help prevent azide build-up.
- Instruments used to read test results must be obtained from or approved by Ellie LLC. Warranty or performance is not guaranteed otherwise.

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

Specimen Requirements

Ellie's BRUCELLA FPA test can be used with individual serum, plasma, or milk samples from cattle.

Serum and Plasma Samples

The test uses only 40 µl of sample per duplicate tests. 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 the blood to clot and separate the 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. Fresh samples should be used for testing. Store samples at 2-8 ℃ for 7 days or freeze it at -20 ℃ if not tested within 72 hours; avoid repeated freezing. Frozen samples should be fully thawed and mixed well before testing.

Milk Samples

Do not test colostrum and milk samples up to 20 days after calving because of nonspecific reactions.

Milk samples should be collected before the milking process. Collect the milk sample (10-50 ml) 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°C.

Clarify milk samples using ClearMilk™ Buffer according to the enclosed procedure. Use 20 µl of milk serum for testing.

Preliminary Steps

Prepare Sample Diluent by mixing one part 25X Sample Diluent with 24 parts of distilled or deionized water.

Ensure the Sample Diluent is free of particulates. Heat up to 37°C to dissolve any crystals. Then, equilibrate to room temperature before use.

Samples must be free of particulates. Centrifuge all samples containing any visible particulates. Hemolyzed samples are acceptable for testing. Lyophilized samples should be reconstituted completely, and frozen samples should be fully thawed and mixed.

Testing Procedure

1. Pipette 20 µl of each sample and control into tubes suitable for the Sentry tube instrument, strip wells for the Sentry Strip Reader, or wells of a microtiter plate for the Sentry microplate instrument. Run Negative Controls in triplicate. Avoid bubbles when pipetting into strips or microtiter plates.

   NOTE: When testing with tubes or Sentry Strips, retest the controls after every 60 samples or 6 strips.
2. Pipette 1 ml of the Sample Diluent into all tubes or pipette 180 µl of Sample Diluent into all microtiter strip wells or the microtiter plate. Mix carefully.
3. Incubate for 3-30 minutes at room temperature.
4. Obtain blank readings for all samples and controls.
5. Add 10 µl of Tracer into all tubes/wells containing controls and samples. Mix carefully.
6. Incubate for 3-5 minutes at room temperature.
7. Obtain mP readings for all samples and controls.

Test Validation

1. The Negative Control must read between 70 and 95 mP.
2. The Positive Control must read between 120 and 250 mP.
3. If the Negative Control is outside of the above range, adjust the instrument to read the mean Negative Control at 80 ±1 mP. For further instructions, consult the instrument manual. Depending on the instrument, this can be done without retesting samples.
4. If the Negative Control is adjusted and the Positive Control is outside of the above range, the test is considered invalid. Please contact technical support at support@ellielab.com.

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

Results & Interpretation

Calculation of ΔmP values

Calculate ΔmP values by subtracting the mean Negative Control mP value from the sample mP value:

ΔmP = (Sample mP - Average Negative Control mP)

Interpretation:

For presumed brucellosis-free zones without vaccination or where RB51 vaccine is used, apply the following cutoffs:

Negative	Suspect	Positive
≤10	10-20	>20

For animals vaccinated with vaccines other than RB51 (e.g., S19, A19 or similar), apply the following cutoffs:

Negative	Suspect	Positive
≤40	40-60	>60

Cutoffs for this group are similar to O.I.E.-proposed FPA cutoffs, published in O.I.E. Manual for Diagnostic Tests and Vaccines.

Positive and suspect samples must be retested in duplicate. If both retests read equal or less than 10 ΔmP, the sample is reported as Negative. If any of the retests are higher than 10 ΔmP, the sample is reported as Positive.

Cutoff values may vary from country to country depending on the different use or vaccination status of animals.

Quality Control

Upon the first use of the test kit, record the ΔmP of the Positive Control. Also, record the mP value of the Negative Control. This information should be systematically recorded and followed. The ΔmP of the Positive Control is a true indication of the condition of the test kit and the instrument. The mP of the Negative Control is an indication of the testing condition and the condition of the Negative Control.

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