

BRUCELLA FPA Milk

BRUCELLA S ANTIBODY TEST KIT, FPA MILK

The BRUCELLA S ANTIBODY TEST KIT, FPA Milk is a qualitative test that uses Fluorescence Polarization technology to determine the presence of antibodies in individual or bulk 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 Milk has been validated for testing individual and bulk milk samples in cattle, sheep and goats. Contact us for more details.

The diagnostic test uses an O-polysaccharide (OPS) extracted from *Brucella abortus* and *Brucella melitensis* 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

Positive Control

2 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.

Sample Diluent

2 x 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

Ready-to-use; proprietary formula that contains O-polysaccharide (OPS) extracted from *Brucella abortus* and *Brucella melitensis* bacteria labeled with fluorescein.
Contains 0.095% sodium azide as a preservative.
Hazard Code: Not classified according to EU regulations.

ClearMilk™ Buffer

20 ml

Ready-to-use; proprietary formula
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
- Pipettors and pipette tips
- Distilled or deionized water
- Microcentrifuge
- 1.5 ml microcentrifuge tubes
- Vortex

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

Storage & Stability

The kit should be stored at 2-8 °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 Milk test can be used with individual or bulk milk samples from cattle, sheep and goats.

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 between 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.

Preliminary Steps

All kit reagents must be equilibrated to room temperature (20-25°C) before use.

Delipidation of milk samples:

There are two ways of delipidating milk samples:

1. Add 1.5 ml of the milk sample into a microcentrifuge tube and centrifuge 5 minutes at 10000 g. The lipid layer will be separated on the top. Carefully collect the layer below the lipid surface.
2. Add 10 - 15 ml of milk into the syringe and leave it vertically overnight with the plunger facing up. The lipids will separate on the top. Eject the skim milk from the syringe.

Clarification of milk samples:

1. Add 1 ml of skim milk sample into a microcentrifuge tube.
2. Add 60 µl of ClearMilk™ Buffer.
3. Mix gently by rocking the tubes immediately after adding the ClearMilk™ Buffer into each tube.
4. Incubate for 5 -15 minutes at room temperature until milk starts to clump.

5. Slowly add 300 µl of Sample Diluent over the top of the clot in each microcentrifuge tube.
6. Centrifuge for 10 minutes in the microcentrifuge at 10000 g.

Testing Procedure

1. Use the first three test tubes for the Negative Control and the fourth for the Positive Control.
2. Pipette 0.7 ml of Sample Diluent into the first four tubes. Add 0.3 ml of distilled or deionized water to each test tube.
3. Add 40 µl of Positive Control into the fourth tube and mix well using vortex.
4. Pipette 1 ml of the Milk serum from the microcentrifuge tube into the test tube.
5. Incubate 3-5 minutes at room temperature.
6. Obtain blank readings of controls and samples.
7. Add 10 µl of Tracer into all tubes containing controls and samples.
8. Mix well using vortex to achieve complete fluid vortex.
9. Incubate for 3-5 minutes at room temperature.
10. Obtain mP readings of controls and samples.

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:

$$\Delta mP = (\text{Sample mP} - \text{Average Negative Control mP})$$

Interpretation:

Individual and Bulk milk samples:

| | |
|------------------------------|---------------------------|
| Negative ≤ 10 | Positive > 10 |
|------------------------------|---------------------------|

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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