

TB ★ FPA

MYCOBACTERIUM BOVIS ANTIBODY TEST KIT, FPA

The MYCOBACTERIUM BOVIS ANTIBODY TEST KIT, FPA is a qualitative test that uses Fluorescence Polarization technology to determine the presence of antibodies against *Mycobacterium bovis* in bovine serum. The presence of antibodies indicates prior infection with *Mycobacterium bovis*.

The diagnostic test uses a fluorescein-labeled peptide with the same amino acid sequence as an epitope region of the *Mycobacterium bovis* bacterium MPB70 protein. The sequence is conserved throughout the *Mycobacterium tuberculosis* complex. A Fluorescence Polarization instrument is used to measure the polarization state of the light emitted by the peptide conjugate.

Polarization is low when no antibodies are present. Polarization increases when antibodies bind to the conjugate.

Kit Contents

Reagents	250 tests	1000 tests
Positive Control	**1.5 ml**	**4.5 ml**
Ready-to-use; bovine positive serum against *Mycobacterium bovis*. Contains 0.095% sodium azide as a preservative. Hazard Code: Not classified according to EU regulations.		
25X Sample Diluent	**50 ml**	**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**	**10 ml**
Ready-to-use; a proprietary formula that contains peptide conjugated 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 microplate instrument
- 4Titude 24 well Assay Plate, solid bottom, black, Product Code: PLATES24
- Precision single micropipettes and tips for volumes between 10 to 1000 µl (e.g., single pipettes 10-100 and 100-1000 µl)
- Deionized, distilled, or RO purified water to make up the Sample Diluent
- Plastic or glass bottles with screw caps and laboratory beakers or Erlenmeyer flasks to make ready-to-use Sample Diluent

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

Storage & Stability

The kit should be stored at 2-8°C. The 25X Sample Diluent can be stored at room temperature. During use, avoid exposing the kit components to temperatures higher than room temperature (up to 25°C). The kit is transported in a cooled box at a temperature between 0-15°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 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 and 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 TB FPA test can be used with serum samples from cattle.

The test uses 200 µl of serum for duplicate tests. Collect the amount of blood required by the blood collection system but no less than 2 ml.

Collect blood aseptically in untreated tubes or serum separator tubes. Allow the blood to clot and separate the serum. Avoid using heavily hemolyzed or contaminated sera. Fresh sera should be used for testing. Store sera at 2-8 °C for 7 days or freeze at -20 °C for 4 months; avoid repeated freezing. Frozen samples should be fully thawed and mixed well before 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 for 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. Use wells A1, B1, and C1 for the Negative Control and D1 for the Positive Control. Run the Negative Control in triplicate.
2. Pipette 100 µl of the Positive Control and each sample into their appropriate wells.
3. Pipette 1000 µl of Sample Diluent into the Negative Control wells (A1 -C1).
4. Pipette 900 µl of Sample Diluent into wells containing the Positive Control and samples. Mix carefully.
5. Incubate for 1 - 60 minutes at room temperature.
6. Obtain blank readings of all samples and controls.
7. Add 10 µl of Tracer into all wells containing controls and samples. Mix carefully.
8. Cover the plate with adhesive foil to protect it from light and incubate for 5 - 60 minutes at room temperature.
9. Obtain mP readings for all samples and controls.

Test Validation

1. The Negative Control must read between 70 and 90 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

Interpreting the results of an antibody assay for tuberculosis should be based on a combination of the epidemiological information of the animal and herd with the detected antibody level. Classifying an animal as positive also depends on the definition of a "positive." For example, a positive animal can be defined as any animal that is positive according to tissue culture or PCR or any animal with lesions found at slaughter. The cutoff will be different based on which definition is selected. To be equivalent to animals testing positive with tissue culture, the TB FPA cutoff is 15. To be equivalent to animals found with lesions, the cutoff is 30. The table below is based on lesions as a definition of a positive animal:

Negative	Suspect	Positive
<15	15-30	>30

Negative ΔmP Results

It is common for a sample to have a lower mP value than the average NC, resulting in a negative ΔmP. Negative ΔmP values are caused by a high concentration of fluorescing molecules in that particular sample. This could be an indicator of sample degradation or naturally high fluorescence in the sample. If the ΔmP result is very low (for example, less than -15) consider obtaining a new sample.

Quality Control

Upon first use of the test kit, record the Delta mP of the Positive Control. This information should be systematically recorded and followed. The Delta mP of the Positive Control is a true indication of the condition of the test kit as well as the instrument.

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