ellie

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Proudly developed in collaboration with a research institute of the Ministry of Agriculture of Brazil, Embrapa Campo Grande.

TB Chimera iELISA (Embrapa Kit)

MYCOBACTERIUM BOVIS ANTIBODY TEST KIT, iELISA

The MYCOBACTERIUM BOVIS ANTIBODY iELISA TEST KIT is a qualitative test using indirect ELISA technology. The test is designed to determine the presence of antibodies against specific peptide sequences of *Mycobacterium bovis* proteins MPB70, MPB83 and ESAT-6 in individual bovine serum samples. The presence of these specific antibodies indicates exposure to *M. bovis*.

Peptide sequences are fused in a chimeric protein and expressed together in a standard *Escherichia coli* recombinant system (cBTB).

The diagnostic test uses microtiter plates that have wells coated with cBTB. Any antibodies present in the sample will bind to the cBTB coated on the plate. After a subsequent wash step, secondary peroxidase-conjugated antibodies (Conjugate) will bind to any immobilized antibodies in the wells. Following another wash step, any bound Conjugate is revealed using a TMB substrate, which produces color in the presence of peroxidase. A microplate reader measures the optical density of the color produced. The amount of color generated is proportional to the amount of specific anti-*M. bovis* antibodies in the animal serum.

Kit Contents

Reagents	2-Plate Kit	5-Plate Kit
Positive Control 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.	**0.5 ml**	**1 ml**
Negative Control Ready-to-use; bovine negative serum against *Mycobacterium bovis*. Contains 0.095% sodium azide as a preservative. Hazard Code: Not classified according to EU regulations.	**0.5 ml**	**1 ml**
Sample Diluent Ready-to-use; proprietary formula contains 0.1% ProClin 300 as a preservative. Hazard Code: Not classified according to EU regulations.	**60 ml**	**120 ml**
100X Conjugate A proprietary formulation containing purified animal antibodies. Hazard Code: Not classified according to EU regulations.	**0.5 ml**	**1 ml**
Substrate Ready-to-use; TMB buffered solution. Hazard Code: Not classified according to EU regulations.	**30 ml**	**70 ml**
10X Wash Buffer Proprietary formula containing 0.1% ProClin 300. Hazard Code: Not classified according to EU regulations.	**100 ml**	**2 x 100 ml**
Conjugate Diluent A proprietary formulation containing animal serum albumin. Hazard Code: Not classified according to EU regulations.	**30 ml**	**70 ml**
cBTB Coated Plate Peptide sequences of MPB70, MPB83 and ESAT-6 proteins are fused in a chimeric protein and expressed together in a standard *Escherichia coli* recombinant system (cBTB). Hazard Code: Not classified according to EU regulations.	**2 plates**	**5 plates**

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 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 pipette 5-50 and 20-200 µl)
- Test tubes or non-antigen-coated transfer plate(s) with flat or U bottom for sample diluting
- Plastic or glass bottles with screw caps, laboratory beakers or erlenmeyer flasks to make ready-to-use Wash Buffer
- Reagent reservoirs to transfer reagents into plates
- ELISA microplate reader or spectrophotometer equipped with 450 nm filter
- Deion-ized, distilled, or RO purified 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.

Kit is transported in a cooled box at a temperature be-tween 0 and 15°C.

Do not use components after the expiration date. Do not mix reagents from different kit serials. Do not expose TMB solution to strong light or any 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 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

disposal of reagents, flush with a large volume of water to help prevent azide build-up.

- Stop Solution contains a dilute acid solution. Use with care to 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 TB iELISA Chimera (Embrapa Kit) test can be performed with serum samples from cattle.

The test uses only 10 µl of serum per single 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 sera. Store sera at 2-8˚C. Freeze sera at -20˚C if not tested within 72 hours; avoid repeated freezing.

Preliminary Steps

Reagent Preparation

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

Take the peptide-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). 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 serum samples 1:20 in Sample Diluent (*e.g.* 10 µl of controls or samples in 190 µl of Sample Diluent). Pipette Controls in duplicate. 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. Transfer 100 µl of prediluted samples and controls into all cBTB Coated Plate test wells. Pipette controls in duplicate.
2. Cover the plate and incubate for 60 minutes at 36-38°C.
3. 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.
4. Dispense 100 µl of Conjugate into each well.
5. Cover the plate and incubate it for 60 minutes at 36-38°C.
6. 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.
7. Dispense 100 µl of Substrate into each well.
8. Incubate for 15 ± 3 minutes at room tempera-ture.
9. Dispense 100 µl of Stop Solution into each well.
10. Read the results at 450 nm on a microplate reader.

Test Validation

- The mean O.D. 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 validation criteria are not met, the test results are in-valid and samples have to be retested

Results & Interpretation

Calculation of Sample to Negative Ratio

S/N Ratio = OD Sample / OD NC

where: OD Sample = OD value of a sample

OD NC = Mean OD value of the Negative Control

Interpretation

Interpreting results of an antibody assay for tuberculosis should be based on a combination of the epidemiological information of the animal and herd, combined with the antibody level detected. Classifying an animal positive is also dependent on the definition of a "positive". For example, a positive animal can be defined as any animal that is positive on tissue culture, or any animal with lesions found at slaughter. The cutoff will be different based on which definition is selected. To be equivalent to animals positive on tissue culture, the TB iELISA Chimera (Embrapa Kit) cutoff would be 4. To be equivalent to animals found with lesions, the cutoff would be 8.

Additional epidemiological information might include how animals are housed- free ranging versus confined. Free ranging animals are less likely to have any reaction on the test.

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