BIONOTE BTB Ab ELISA 2.0

■ Principle of the Test

The BIONOTE BTB Ab ELISA 2.0 is a direct Enzyme Linked Immunosorbent Assay for the qualitative detection of antibody against *Mycobacterium bovis* in serum.

The BIONOTE BTB Ab ELISA 2.0 contains Microplates, which are pre-coated with purified *M. bovis* antigen on the well. For testing, ELISA Microplates coated with the antigen are incubated with an equal mixture of serum and *M.Bovis* antigen-HRP conjugate for 60 minutes at 37 ℃. During the incubation, *M. bovis* antibodies present in the test sample bind to purified *M. bovis* antigen pre-coated in the well and conjugate. Following this incubation, all unbound material is removed by aspiration and washing before adding a TMB Substrate. The residual enzyme activity found in the well will thus be directly proportional to the conjugate concentration in the sample and evidenced by incubating the solid phase with a TMB Substrate. The reaction is stopped by the addition of the Stop Solution and colorimetric reading should be performed by using a spectrophotometer at 450 ㎚ with a reference wavelength at 620 ㎚.

The specially selected *M. bovis* antigens are used as capture material in the test. These enable the BIONOTE BTB Ab ELISA 2.0 to identify *M. bovis* antibodies in the sample, with a high degree of accuracy.

■ Materials Provided

BIONOTE BTB Ab ELISA 2.0 contains following items to perform the assay.

1) Anigen coated Microplate (1)
2) Negative Control (2)
3) Positive Control (3)
4) 10X Washing Solution (4)
5) Enzyme Conjugate (5)
6) TMB Substrate (6)
7) Stop Solution (7)
8) Adhesive Plate Sealer (8)
9) Instructions for use (9)

■ Materials required, but not provided

- Precision pipettes or multiple delivery pipetting devices suitable for delivering 10 to 1000 µl
- Disposable pipette tips
- 500 ml graduated cylinder for Washing Solution
- 96-well plate reader
- Distilled or deionized water
- Vortex mixer

■ Precautions

In order to obtain reproducible results, the following rules must be observed:

1) For *in vitro* diagnostic use only.
2) Store the components at 2~8 ℃ right after use. Do not reuse Microplate (1) or pour reagents back into their original bottles once dispensed.
3) Do not use reagents after the expiry date.
4) Do not mix reagent of different lots.
5) Wear the gloves when you handle the potentially infectious materials. After handling, wash hands with sanitizers.
6) Haemolytic samples should be centrifuged before use to avoid interference by cellular constituents.
7) Clean the ELISA equipment and test area before performing the assay. It can affect to test result.
8) Blood corpuscle in samples may also give non-specific reaction.
9) TMB Substrate (6) and Stop Solution (7) should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident rinse thoroughly with running water.
10) Dispose of containers and residues safely in accordance with national and local regulations.

■ Collection and Storage of Sample

1) Fresh serum samples can be used for this assay. Hemolyzed or contaminated samples may give erroneous results. Blood corpuscle in samples may also give a non-specific reaction.
2) Mix samples thoroughly by gentle inversion. If necessary, any visible particulate matter in the samples should be removed by low-speed centrifugation.
3) Serum samples should be inactivated at 56 ℃ for 30 min.
4) Serum samples should be stored at 2~8 ℃. For longer storage(more than 3 days), freeze the samples at -20 ℃ or below. Avoid repeated freezing and thawing.
5) Hemolytic or contaminated samples must be avoided.
6) Sodium azide in the sample affects to test result.

■ Preparation of Reagent and Samples

1) Unused Microplate (1) must be sealed with silica gel in enclosed sealing bag and stored at 2~8 ℃.
2) **10X Washing Solution (4)**: The concentrated 10X Washing Solution (4) must be diluted 1 : 9 with distilled/deionized water before use. (i.e. add 100 ㎖ of 10X Washing Solution (4) to 900 ㎖ of distilled/deionized water) and mix well. If undissolved crystals are present, re-suspend the solution by warming the bottle at 37 ℃ for few minutes. Store at 2~30 ℃.

■ Procedure of the Test

1) Allow all reagents and samples to come to room temperature (18~25 ℃) for 30 minutes before use.
2) Prepare the strip wells for Negative Control (2) 2 wells, Positive Control (3) 2 wells and each of the samples to each well.
3) Add 50 ㎕ of Negative Control (2), Positive Control (3) to 2 wells correspondingly, and 50 ㎕ of samples to each well.

4) Add 50 ㎕ of Enzyme Conjugate (5) to each well.
5) Cover the wells with Plate Sealer (8) and incubate the wells at 37 ℃ for 60 minutes.
6) Wash the wells 6 times with 350 ㎕ of diluted Washing Solution. Aspirate all liquid from the wells.
7) Add 100 ㎕ of TMB Substrate (6) to each well.
8) Cover the wells with Plate Sealer (8) and incubate the wells at room temperature (18~25 ℃) for 15 minutes in the dark.
9) Add 100 ㎕ of Stop Solution (7) to each well. Mix by gentle shaking.
10) Read the absorbance of the wells with a bichromatic spectrophotometer at 450 ㎚ with reference wavelength at 620 ㎚. Reading must be completed within 30 minutes. from the end of assay.

■ Interpretation of the Result

1) Test validation

① The mean OD of Negative Control(OD_{NCx}) should be below 0.150.
② The mean OD of Positive Control(OD_{PCx})should be above 1.500.
③ If either of these values are not of range, BIONOTE BTB Ab ELISA 2.0 test should be considered invalid and the samples should be retested.

2) Calculation of S/P value

$$S/P = \frac{(OD_{sample} - OD_{NCx})}{(OD_{PCx} - OD_{NCx})}$$

3) Interpretation of result

After calculating the S/P value, the Positive and Negative value should be determined based on the following S/P criteria.

① Positive: S/P of sample ≥ 0.5
② Negative: S/P of sample < 0.3
③ Suspicious-positive: 0.3 ≤ S/P of sample < 0.5

- A final diagnosis cannot be made solely based on the results of this product because this product cannot completely exclude the possibility of false positive or false negative results due to various factors. It must be used by a professional veterinarian, and the final diagnosis must be made based on the results of this product, other test results, and clinical findings.

■ Limitations and Interferences

1) The test procedure, precautions and interpretation of results sections for this test kit must be complied when testing.

■ Storage and Stability

1) The BIONOTE BTB Ab ELISA 2.0 kit should be stored at 2~8 ℃. This test kit is stable through the expiration date printed in the package and in the label of each material/reagent as unopened state.

2) Stability of once opened materials/reagents

Reagent	State	Storage	Stability
Working washing solution	1:9 diluted	2~30 ℃	1 week

■ Packaging Unit

Reagent \ Volume	96 Tests/Kit	480 Tests/Kit	960 Tests/Kit
Antigen Coated Microplate (1)	1 plate	5 plates	10 plates
Negative control (2)	1 vial (0.5 ㎖/vial)	1 vial (2.5 ㎖/vial)	1 vial (4.5 ㎖/vial)
Positive control (3)	1 vial (0.5 ㎖/vial)	1 vial (2.5 ㎖/vial)	1 vial (4.5 ㎖/vial)
10X Washing Solution (4)	1 bottle (50 ㎖/bottle)	1 bottle (250 ㎖/bottle)	2 bottles(250 ㎖/bottle)
Enzyme Conjugate (5)	1 bottle (8 ㎖/vial)	1 bottle (40 ㎖/vial)	1 vial (80 ㎖/vial)
TMB Substrate (6)	1 bottle (12 ㎖/vial)	1 bottle (60 ㎖/vial)	1 bottle (120 ㎖/vial)
Stop Solution (7)	1 bottle (15 ㎖/bottle)	1 bottle (80 ㎖/bottle)	1 bottle (150 ㎖/bottle)
Adhesive Plate Sealer (8)	2 EA	10 EA	20 EA
Instructions for use (9)	1 EA	1 EA	1 EA

■ Bibliography of suggested reading

1) Sang-Nae Cho. Expression of the MPB70 Protein of *Mycobacterium bovis* and Use in the Serodiagnosis of Bovine Tuberculosis. Kor.J.Vet.Publ,Vol. 22, No. 2, 1998
2) Manual of diagnostic Tests and Vaccines for Terrestrial Animals. 5th edition. 2004. Part 2. Chapter 2.4.7 'Bovine Tuberculosis'

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