Mycobacterium bovis Antibody ELISA

AniGen BTB Ab ELISA

1. Principle of the Test

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

The AniGen BTB Ab ELISA contains a microplate, which is pre-coated with purified BTB antigen on the well. For testing, ELISA plates coated with the antigen are incubated with an equal mixture of serum and conjugate (1: 100 dilution in the conjugate diluents) for 60 minutes at 37℃. During the incubation, *M. bovis* antibodies present in 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 substrate solution. The residual enzyme activity found in the well will thus be directly proportional to the conjugate concentration in specimens and evidenced by incubating the solid-phase with a substrate solution. The reaction is stopped by addition of the stopping solution and colorimetric reading should be performed by using a spectrophotometer at 450 ㎚ with reference wavelength at 620 ㎚.

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

2. Materials Provided (480Tests/Kit)

AniGen BTB Ab ELISA contains following items to perform the assay.

1) Microplate coated of purified BTB Ag ①: 5 plates (96wells/plate) configured in twelve 1x8 strips.
2) Standard negative control serum ② : 1 vial (2.5 ㎖/vial) of normal bovine serum treated with calcium. Sodium azide (0.01%) added as preservatives.
3) Standard strong positive control serum ③: 1 vial (2.5 ㎖/vial) of anti-BTB strong positive bovine serum treated with calcium. Sodium azide (0.01%) added as preservatives.
4) Washing solution (10X concentrated) ④: 1 bottle (250 ㎖/bottle) of PBS-Tween 20.
   Preservative : Thimerosal (0.01%)
   ***Note**: Before use, take one bottle content, and then fill-up to 1,000 ㎖ with distilled water. In presence of undissolved crystals, re-suspend the solution by placing the bottle at 37 ℃ for a few minutes.
5) Enzyme conjugate : (101X concentrated)⑤: 1 vial (1.2 ㎖/vial) of *M. bovis* antigen –HRP.
   Preservative : Proclin (0.06%)
6) Enzyme conjugate diluent⑥: 1 vial (40 ㎖/vial) of phosphate buffered saline.
   Preservative : Proclin (0.06%)
7) Substrate (Ready to use) ⑦ : 1 bottle (60 ㎖/vial) of tetramethyl-benzidine with citrate-phosphate buffer containing H_2O_2.
8) Stopping solution ⑧: 1 bottle (80 ㎖/bottle) of 1N sulfuric acid. Ready for use.
9) Adhesive plate sealer: 10 EA
10) Instructions for use.

3. Precautions

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

1) For in vitro diagnostic use only.
2) Do not mix reagent of different lots.
3) Use thoroughly clean glassware, free from contamination of metal ion or oxidating substances.
4) Use disposable gloves while handling potentially infectious material and performing the assay.
5) Substrate and stopping solution should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident rinse thoroughly with running water.

4. Specimen Collection and Storage

1) Centrifuge whole blood to get serum.
2) If specimens are not immediately tested they should be refrigerated at 2 ~ 8℃. For storage periods greater than three days, freezing is recommended. They should be brought to room temperature prior to use.
3) Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

5. Reagent preparation

1) Allow all reagents to come to room temperature (18~25℃) before use.
2) **Enzyme conjugate (101X concentrated)**: The concentrated enzyme conjugate ⑤ must be diluted 1 : 100 with enzyme conjugate diluents⑥ before use. (i.e. add 10 ㎕ of enzyme conjugate ⑤ to 1ml of conjugate diluent ⑥ and mix well).
3) **Washing solution (10X concentrated)**: The concentrated washing solution④ must be diluted 1 : 9 with distilled/deionized water before use. (i.e. add 100 ㎖ of Washing solution④ to 900 ㎖ of distilled water) and mix well. If undissolved crystals are present, re-suspend the solution by warming the bottle at 37℃ for few minute.

6. Procedure of the Test

1) Prepare the strip wells for negative control serum 3 wells, positive control serum 3 wells and each of the samples to each well.
2) Add 50 ㎕ of each of the primary positive, negative control serum to 3 wells, and 50 ㎕ of each of the samples to each well.
3) Add 50 ㎕ of *M. bovis* antigen-HRP(1:100 dilution in the conjugate diluent) to each well.
4) Cover the microplate with adhesive plate sealer and mix well on vibrating mixer. Mixing is very I mportant to get reproducible results
5) 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 mixed substrate (Ready to use) to each well.
8) Incubate the wells for 15 minutes at room temperature (18~25℃).
9) Add 100 ㎕ of stopping solution to each well.
10) Read the absorbance of the wells with a bichromatic spectrophotometer at 450 ㎚ with reference wavelength at 620 ㎚. Reading must be completed within 1 hour from the end of assay.

7. Interpretation of the Test

1) Test validation
   ① The OD value of standard negative control serum should be below 0.150.
   ② The OD value of standard positive control serum should be above 1.500.
   ③ If either of these values are not of range, AniGen BTB Ab ELISA should be considered invalid and the samples should be retested.

2) S/P

$$S/P = \frac{\text{(Sample O.D – Average OD of Negative control serum)}}{\text{(Average OD of Positive control serum – Average OD of Negative control serum)}}$$

3) Interpretation of serum
   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 < 0.5

8. Limitations and Interferences

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

2) Samples

① Samples containing sodium azide do not affect the test result.

② Pasteurized sera samples (no less than 10 hours at 60℃) may lead to diminished reactivity. Therefore should not be used.

③ Heat-inactivated sera samples (1 hour at 56℃) do not impair the test.

④ Anticoagulants such as heparin, EDTA, and citrate do not affect the test result.

⑤ Haemolytic samples should be centrifuged before use to avoid interference by cellular constituents.

⑥ Rheumatoid factors can lead to elevated reactivity if contained in the samples.

⑦ Lipaemic and icteric samples do not impair the test results.

⑧ This test kit detects antibody to *M. bovis* antibody in serum samples and thus is useful as a screening procedure.

⑨ Failure to add specimen in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of infection.

9. Storage and stability

1) The AniGen BTB Ab ELISA 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

Material/Reagent	State	Storage	Stability
Diluted washing solution	1:9 diluted	2~8℃ Room temp(18~25℃).	1 weeks
Enzyme conjugate diluent	1:100 diluted	Room temp(18~25℃). Closed container, protected from light.	8 hours

10. Packaging Unit : 96Test/Kits, 480Tests/kit, 960 Tests/kit

11. Precision

Within-run and between-run precisions have been determined by testing 10 replicates of three specimens : standard negative serum, standard positive serum. The C.V(%) of negative , positive values were within 10%

12. 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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