BIONOTE Brucella Ab C-ELISA

■ Principle of the Test

Brucellosis is a highly contagious bacterial disease, caused by *Brucella* spp. leading to late-term abortion and infertility in cattle. The disease is also a serious zoonosis, causing undulant fever in humans.

The BIONOTE Brucella Ab C-ELISA is a competitive Enzyme-Linked Immunosorbent Assay(ELISA) for the qualitative detection of antibody against *B. abortus, B. melitensis*, and *B. suis* in serum or plasma. This assay is able to differentiate between vaccination antibodies (S19) and those that result from *Brucella* infection.

The BIONOTE Brucella Ab C-ELISA contains a microplate, which is pre-coated with non-infectious Brucella antigen on the well. For testing, BIONOTE Brucella Ab C-ELISA plates are incubated with an equal mixture of sample and monoclonal antibody-Biotin conjugate for 30 minutes at the room temperature. During first incubation, Brucella antibodies(if present in the test sample) and anti-Brucella LPS monoclonal antibody bind competitively to the antigens in the well. After incubation, unbound materials are removed by aspiration and washing. Streptavidin-HRP conjugate added subsequently forms a complex with the monoclonal brucella antibody-Biotin conjugate. Unbound materials are removed by aspiration and washing again and TMB substrate solution is added. The enzyme activity found in the well will thus be directly inversely proportional to the Brucella antibody concentration in samples. The reaction is stopped by addition of the stop solution and colorimetric reading should be performed by using a spectrophotometer at 450 ㎚ with reference wavelength at 620 ㎚.

■ Materials provided

BIONOTE Brucella Ab C-ELISA contains following items to perform the assay.

1) Antigen Coated Microplate (1)
2) Negative Control (2)
3) Strong Positive Control (3)
4) Weak Positive Control (4)
5) 5X Sample Diluent (5)
6) 3X Amplification Conjugate(Freeze dried) (6)
7) 20X Washing Solution (7)
8) 101X Enzyme Conjugate (8)
9) Conjugate Diluent (9)
10) TMB Substrate (10)
11) Stop Solution (11)
12) Adhesive Plate Sealer (12)
13) Instructions for use (13)

■ Materials required, but not provided

- Precision pipettes
- Disposable pipette tips
- Distilled/deionized water
- Wash bottle
- Bichromatic spectrophotometer

■ 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 microwells or pour reagents back into their original bottles once dispensed.
3) Do not intermix components from kits with different batch numbers.
4) Do not use reagents after the expiry date.
5) Do not reuse containers and residues to avoid contamination in reagents due to samples or other reagents.
6) Handle all reagents and samples as biohazardous materials.
7) Use fresh samples. Hemolyzed or contaminated samples may give erroneous results.
8) Remove the red blood cell in samples clearly. It may give non-specific reaction.
9) Wear the gloves when you handle the potentially infectious materials. After handling, wash hands with sanitizers.
10) Keep all reagents away from skin and eyes. If exposure should occur, immediately rinse with fresh cold water.
11) Dispose of containers and residues safely in accordance with national and local regulations.
12) TMB Substrate and stop solution can cause irritation or burns to the skin and eyes. In case of accident, rinse immediately with fresh cold water.

■ Collection and Storage of Sample

[Serum or Plasma]

1) Fresh serum or plasma samples should be used for this assay.
2) Hemolyzed or contaminated samples may give erroneous results.
3) If samples are not immediately tested, they should be refrigerated at 2~8 ℃. For longer storage, freeze the samples at -20 ℃ or below. Avoid repeated freezing and thawing.

■ Preparation of Reagents

1) Unused microplate wells must be sealed with silica gel in enclosed sealing bag and stored at 2~8 ℃.
2) **101X Enzyme Conjugate** : Dilute enzyme conjugate 1 : 100 with enzyme conjugate diluents before use. (e.g., add 10 ㎕ of enzyme conjugate to 1 ㎖ of conjugate diluent and mix well.)
3) **3X Amplification Conjugate (Freeze dried)** : 3X Amplification Conjugate must be dissolved with 3.0 ㎖ of conjugate diluent.
4) **5X Sample Diluent :** Dilute 5X Sample Diluent 1 : 4 with distilled/deionized water before use. (e.g., add 10 ㎖ of 5X Sample Diluent to 40 ㎖ of distilled water and mix well.)
5) **20X Washing Solution :** Dilute 20X Washing Solution 1 : 19 with distilled/deionized water before use. (e.g., add 25 ㎖ of 20X Washing Solution to 475 ㎖ of distilled water and mix well. If undissolved crystals are present, re-suspend the solution by warming the bottle at 37 ℃ for few minutes.)
6) **Stability of Prepared Reagent**

Reagents	State	Storage	Stability
Diluted Amplification Conjugate	Once prepared	2 ~ 8 ℃	7 days
Diluted Enzyme Conjugate	Once prepared	2 ~ 8 ℃	8 hours
Diluted Washing Solution	Once prepared	Room temp.	1 week

■ Procedure of the Test

1) Allow all reagents and samples to come to room temperature (18~25 ℃) before use.
2) Dilute controls and samples 1:9 with sample diluent(e.g., by diluting 10 ㎕ of sample with 90 ㎕ of sample diluent).
3) Add **50 ㎕ of diluted samples and controls** into each sample well.
   - NC; 3 wells, WPC; 2 wells, SPC; 2 wells,
4) Add **50 ㎕ of diluted amplification conjugate** into wells of samples and controls.
5) Shake the plate(s) gently and cover the plate(s) with an adhesive plate sealer. Incubate the wells for **30 minutes at room temperature (18 ~ 25 ℃).**
6) Aspirate all liquid from the wells and rinse the wells five times with 350 ㎕ of diluted washing solution. Remove any remaining washing solution by inverting the plate and blotting it against a clean paper towel.
7) Add **100 ㎕ of diluted enzyme conjugate** to each well.
8) Cover the wells with plate sealer and incubate for **30 minutes at room temperature (18 ~ 25 ℃).**
9) Wash the wells as described above in **'6)'**.
10) Add **100 ㎕ of TMB substrate** to each well.
11) Cover the wells with plate sealer and incubate for **15 minutes at 18 ~ 25 ℃ in the dark.**

12) Add **100 ㎕ of stop solution** to each well.
13) Read the absorbance values of the wells at 450 ㎚ in a bichromatic spectrophotometer(with reference wavelength at 620 ㎚) right after from the end of assay, within 30 minutes.

■ Interpretation of the Result

1) Test validation

① The OD value of **negative control** (average of 3 wells) should be **above 1.500.**
② The PI value of **strong positive control** (average of 2 wells) should be **above 90.**
③ The PI value of **weak positive control** (average of 2 wells) should be ranged **40~70.**

2) Calculation of Percentage Inhibition(PI) Value

PI Value = [1-(OD_{sample} / mean of OD_{NC})] x 100

3) Interpretation of the result

① The result should be determined based on the following **PI criteria :**

Result	PI criteria
Negative	PI < 30
Positive	PI ≥ 30

② If result is positive, sample should be tested again in duplicates. If 1 well or more is(are) positive again, the sample is determined as positive.
③ In cattle, this test can discriminate between vaccination antibodies (S19) and those that result from field infection. Samples from S19-vaccinated cattle are negative, while samples from *Brucella*-infected cattle are positive.
④ This assay has no cross-reaction with antibodies against other gram-negative bacteria.
⑤ A definitive clinical diagnosis should be made by the veterinarian after all clinical, vaccination history and laboratory findings have been evaluated.

■ Limitations and interferences

1) For *in vitro* diagnostic use only.
2) Failure to add the sample during the test could result in false-negative results. Repeat testing should be considered where there is clinical suspicion of infection.
3) Other clinically available tests are required if questionable results are obtained. As other diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test. It is recommended the diagnosis decision is made by the clinician after all clinical and laboratory findings have been evaluated.

■ Stability and Storage

The ELISA kit should be stored at 2~8 ℃. This test kit is stable through to the expiration date printed in the package and in the label of each material / reagent in an unopened state. The shelf life is 12 months.

Reagent	State	Storage	Stability
Antigen Coated Microplate	Unopened	2~8 ℃	12 months
Antigen Coated Microplate	Opened and Resealed	2~8 ℃	3 months
Reagents	Unopened	2~8 ℃	12 months
Reagents	Opened and Resealed	2~8 ℃	3 months

■ Packaging unit

Reagent \ Volume	96 Tests/Kit	480 Tests/Kit	960 Tests/Kit
Antigen Coated Microplate (1) (8wells x 12 strip)	1 plate	5 plates	10 plates
Negative Control (2)	0.2 ㎖/vial x 1	0.5 ㎖/vial x 1	1.0 ㎖/vial x 1
Strong Positive Control (3)	0.2 ㎖/vial x 1	0.5 ㎖/vial x 1	1.0 ㎖/vial x 1
Weak Positive control (4)	0.2 ㎖/vial x 1	0.5 ㎖/vial x 1	1.0 ㎖/vial x 1
5X Sample Diluent (5)	25 ㎖/bottle x 1	125 ㎖/bottle x 1	250 ㎖/bottle x 1
3X Amplification Conjugate (6) (Freeze dried)	3 ㎖/vial x 2	3 ㎖/vial x 10	3 ㎖/vial x 20
20X Washing Solution (7)	50 ㎖/bottle x 1	250 ㎖/bottle x 1	250 ㎖/bottle x 2
101X Enzyme Conjugate (8)	0.3 ㎖/vial x 1	1.2 ㎖/vial x 1	2.5 ㎖/vial x 1
Conjugate Diluent (9)	25 ㎖/bottle x 1	125 ㎖/bottle x 1	250 ㎖/bottle x 1
TMB Substrate (10)	12 ㎖/bottle x 1	60 ㎖/bottle x 1	60 ㎖/bottle x 2
Stop Solution (11)	15 ㎖/bottle x 1	80 ㎖/bottle x 1	200 ㎖/bottle x 1
Adhesive Plate Sealer (12)	2 ea	10 ea	20 ea
Instructions for use (13)	1 ea	1 ea	1 ea

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