Bovine Brucella Antibody ELISA 2.0

BIONOTE B. Brucella Ab ELISA 2.0

■ Explanation of the Test

Bovine brucellosis is a highly contagious bacterial disease, almost exclusively caused by *Brucella abortus* causing late term-abortion and infertility in cattle. The disease is also a serious zoonosis, causing undulant fever in humans.

The BIONOTE B. Brucella Ab ELISA 2.0 is an indirect Enzyme Linked Immunosorbent Assay (ELISA) for the qualitative detection of *Brucella abortus* antibody in serum, plasma or milk.

The BIONOTE B. Brucella Ab ELISA 2.0 contains a Microplate, which is pre-coated with non-infectious Brucella antigens on the well. During first incubation, Brucella antibodies (if present in the test sample) bind to the antigens in the well. Unbound materials are removed by aspiration and washing. The added enzyme conjugate subsequently forms complexes with the Brucella antibodies. Unbound materials are removed by aspiration and washing again and TMB Substrate is added. The residual enzyme activity found in the well will thus be directly proportional to the Brucella antibody concentration in samples. The reaction is stopped by the addition of the Stop Solution and colorimetric reading should be performed by using a spectrophotometer at 450 ㎚ with reference wavelength at 620 ㎚.

■ Materials Provided

Antigen coated Microplate (1)	96 well plate configured in twelve 1x8 strips.
Negative Control (serum) (2)	Normal bovine serum, Proclin (0.05 %)
Strong Positive Control (serum) (3)	Strong positive bovine serum against B.Brucella antibody, Proclin (0.05 %)
Weak Positive Control (serum) (4)	Weak positive bovine serum against B.Brucella antibody, Proclin (0.05 %)
Negative Control (milk) (5)	Normal milk, Proclin (0.01 %)
Positive Control (milk) (6)	Positive bovine milk, Proclin (0.01 %)
5X Sample Diluent (7)	Phosphate buffered saline, Proclin (0.01 %)
10X Washing Solution (8)	PBS-Tween 20, Proclin (0.05 %) **Note**: If undissolved crystals are present, re-suspend the solution by placing the bottle at 37 ℃ for few minutes.
101X Enzyme Conjugate (9)	Anti-bovine IgG–HRP, BSA, Proclin (0.05 %)
Conjugate Diluent (10)	Phosphate buffered saline, Proclin (0.05 %)
TMB Substrate (11)	Tetramethyl-benzidine with citrate-phosphate buffer containing H_2O_2.
Stop Solution (12)	1N sulfuric acid. Ready to use.
Adhesive Plate Sealer (13)	
Instructions for use (14)	

■ Materials required, but not provided

- Precision pipets
- Disposable pipet 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 blood corpuscle 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 (11) and Stop Solution (12) can cause irritation or burns to the skin and eyes. In case of accident, rinse immediately with fresh cold water.
13) If the color reaction starts to appear from one part of the well, it is not considered negative.

■ 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.
4) For serum samples, the heat-inactivation (56 ℃, 30 minutes) does not affect the judgement result.

[Milk]

1) Milk samples must be centrifuged for 15 minutes at 2,000 Xg to remove the lipid layer.
2) 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.
3) A pooled milk sample can be used for testing.

■ Preparation of Reagent

1) Unused microplate wells must be sealed with silica gel in enclosed sealing bag and stored at 2~8 ℃.
2) **101X Enzyme Conjugate (9):** Dilute 101X Enzyme Conjugate 1 : 100 with Conjugate Diluent before use. (e.g. add 50 ㎕ of 101X Enzyme Conjugate to 5ml of Conjugate Diluent and mix well.)
3) **5X Sample Diluent (7):** Dilute 5X Sample Diluent 1 : 4 with distilled/deionized water before use. (e.g. add 100 ㎖ of 5X Sample Diluent to 400 ㎖ of distilled water and mix well.)
4) **10X Washing Solution (8):** Dilute 10X Washing Solution 1 : 9 with distilled/deionized water before use. (e.g. add 50 ㎖ of 10X Washing Solution to 450 ㎖ of distilled water and mix well. If undissolved crystals are present, re-suspend the solution by warming the bottle at 37 ℃ for few minutes.)
5) The storage and stability conditions of the prepared test reagents are as follows:

Reagent	Storage	Stability
Diluted Sample Diluent	2~30℃	1 week
Diluted Washing Solution	2~30℃	1 week
Diluted Enzyme Conjugate	2~8℃	8 hours

■ Procedure of the Test

1) Allow all reagents and samples to equilibrate at room temperature (18~25℃) for 30 minutes and shake them gently before use.
2) Take out only the number of wells required for the test. Then seal the remaining wells back using the silver foil with silica gel and store at 2~8℃.
3) Dilute serum or plasma samples 1 : 49 with Sample Diluent (e.g., by diluting 10 ㎕ of sample with 490 ㎕ of diluted Sample Diluent).
   ***NOTE:** Do Not Dilute Negative Control (NC) (2), Strong Positive Control (SPC) (3), Weak Positive Control (WPC) (4) serum.
4) Add **100 ㎕ of prepared sample (serum or plasma; diluted, milk; not diluted)** into each sample well.
5) Add **100 ㎕ of Controls (not diluted) into appropriate wells.**
   - NC (serum) (2); 2 wells, WPC (serum)(4); 2 wells, SPC (serum)(3); 3 wells
   - NC (milk) (5); 2 wells, PC (milk) (6); 3 wells

6) Cover the Microplate (1) with Adhesive Plate Sealer (13) and mix well on vortex mixer. Incubate the wells **for 60 minutes at 37 ℃.**
7) Prepare the diluted Washing Solution in accordance to **'preparation of Reagent – '4)''**.
8) 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.
9) Prepare the diluted Enzyme Conjugate in accordance to **'Preparation of reagents – '2)''**.
10) Add **100 ㎕ of diluted Enzyme Conjugate** to each well.
11) Cover the wells with Plate Sealer (13) and incubate for **30 minutes at 37 ℃.**
12) Wash the wells as described above in Step 8).
13) Add **100 ㎕ of TMB substrate (11)** to each well.
14) Cover the wells with Plate Sealer (13) and **incubate for 15 minutes at 18 ~ 25℃ in the dark.**
15) Add **100 ㎕ of Stop Solution (12)** to each well. Mix by gentle shaking.
16) Read the absorbance values of the wells at 450 nm in a bichromatic spectrophotometer (with reference wavelength at 620 nm) right after from the end of assay, within 30 minutes.

■ Interpretation of the Result

1) Test validation

[Serum & Plasma]

① The OD value of NC (serum) (average of two wells) should be ranged from -0.005 ~ 0.2000.
② The OD value of SPC (serum) (average of three wells) should be above 1.000
③ The OD value of WPC (serum) should be above 0.500

[Milk]

① The OD value of NC milk should be ranged from 0.000~0.2000.
② The OD value of PC milk should be above 1.000

2) Calculation of Percent Positivity(%P) value

[Serum & Plasma]

$$\%P = \frac{OD_{samples}}{\text{Average } OD_{SPC\ serum}} \times 100$$

[Milk]

$$\%P = \frac{OD_{samples}}{\text{Average } OD_{PC\ Milk}} \times 100$$

3) Interpretation of Result

① The result should be determined based on the following %P criteria.

	Serum / Plasma	Milk
Positive	≥ 25	≥ 15
Negative	< 25	< 15

② Negative: Samples with a %P value less than the criterion value are considered negative.
③ Positive: Samples with a %P value greater than or equal to the criterion value are considered positive.
④ If the result is positive, retest the sample in duplicate. If one or more wells show a positive response, then the result is considered positive.
⑤ Professional veterinarian should make a final diagnosis based on the results of this product, other test results and clinical findings.

4) Example

OD_{sample} = 1.870, average $OD_{SPC\ serum}$= 2.124
%P value = (1.870 / 2.124) x 100 = 88
→ This sample is regarded as positive to bovine brucellosis.

■ Limitations and Interferences

1) The test procedure, precautions and interpretation of results sections for this test kit must be followed closely when testing.
2) This test kit detects antibodies against brucella specific antigen in serum and plasma samples and thus is useful as a screening procedure.
3) Failure to add sample in the procedure could lead to a false negative result. Repeat testing should be considered where there is clinical suspicion of infection.

■ Stability and Storage

1) All reagents should be stored at 2~8℃. Do not freeze.
2) Shelf life is 12 months. Use all reagents before the expiry date on the kit.

■ 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 (serum) (2)	1 vial (1.0 ㎖/vial)	1 vial (4.0 ㎖/vial)	2 vials (4.0 ㎖/vial)
Strong Positive Control (serum) (3)	1 vial (1.0 ㎖/vial)	1 vial (4.0㎖/vial)	2 vials (4.0 ㎖/vial)
Weak Positive Control (serum) (4)	1 vial (1.0 ㎖/vial)	1 vial (4.0㎖/vial)	2 vials (4.0 ㎖/vial)
Negative Control (milk) (5)	1 vial (1.0 ㎖/vial)	1 vial (4.0 ㎖/vial)	2 vials (4.0 ㎖/vial)
Positive Control (milk) (6)	1 vial (1.0 ㎖/vial)	1 vial (4.0 ㎖/vial)	2 vials (4.0 ㎖/vial)
5X Sample Diluent (7)	1 bottle (25 ㎖/bottle)	1 bottle (125 ㎖/bottle)	1 bottle (250 ㎖/bottle)
10X Washing Solution (8)	1 bottle (50 ㎖/bottle)	1 bottle (250 ㎖/bottle)	2 bottles(250 ㎖/bottle)
101X Enzyme Conjugate (9)	1 vial (0.3 ㎖/vial)	1 vial (1.2 ㎖/vial)	1 vial (2.5 ㎖/vial)
Conjugate Diluent (10)	1 bottle (15 ㎖/vial)	1 bottle (80 ㎖/vial)	1 bottle (150 ㎖/vial)
TMB Substrate (11)	1 bottle (12 ㎖/vial)	1 bottle (60 ㎖/vial)	2 bottles (60 ㎖/vial)
Stop Solution (12)	1 bottle (15 ㎖/bottle)	1 bottle (80 ㎖/bottle)	1 bottle (150 ㎖/bottle)
Adhesive Plate Sealer (13)	2 EA	10 EA	20 EA
Instructions for use (14)	1 EA	1 EA	1 EA

Doc. No.: I4301-10E
Revised date: Jun. 07, 2022

Manufactured by BIONOTE
BioNote, Inc.
22 Samsung1ro 4-gil, Hwaseong-si, Gyeonggi-do 18449, Republic of Korea
TEL: 82-31-211-0516 I FAX : 82-31-8003-0618 I www.bionote.co.kr