VDPro® Brucella AB ELISA

CAT. NO. EB-BRU-01

MEDIAN Diagnostics

GENERAL DESCRIPTION

VDPro® Brucella AB ELISA is a Brucella abortus specific somatic antigen based two-step Indirect ELISA for the detection of the specific antibodies induced by infection of Brucella abortus in cattle.

The specimen is added to the Brucella abortus specific antigen coated plate followed by the incubation of the secondary antibodies(HRPO Anti-Bovine IgG Conjugate). If the Brucella abortus specific antibodies are present in the specimen, the chromogenic signal by substrate will be appeared.

The result is calculated by using the sample to positive control ratio(S/P ratio).

KIT COMPONENTS

	Reagents	480 tests
①	Brucella Antigen Coated Plate	5 plates
②	10X Washing Buffer	240㎖ X 1
③	Dilution Buffer	240㎖ X 1
④	HRPO Anti-Bovine IgG Conjugate	70㎖ X 1
⑤	Positive Control, PC	7.0㎖ X 1
⑥	Negative Control, NC	7.0㎖ X 1
⑦	TMB Substrate	70㎖ X 1
⑧	Stop Solution	40㎖ X 1
⑨	Sealing Film	5 sheets
⑩	Introduction Manual	1 copy

PREPARATION

1. All reagents must be allowed to come to room temperature (20~25℃) before use. Mix reagents by gentle swirling.
2. 1X washing buffer preparation
   1) Shake 10X Washing Buffer(②) gently.
   2) Dilute 1 part of 10X Washing Buffer(②) with 9 parts of deionized water. The diluted 1X washing buffer is stable for 2 weeks at room temperature (20~25℃).
3. Sample preparation
   1) Serum or plasma samples may be used in this assay.
   2) Use fresh samples for the best result. Samples can be stored at 2~8℃ for less than 3 days or −20℃ for a longer period. Do not freeze and thaw samples repeatedly. **Sera with hemolysis or bacterial contamination are not suitable for the analysis!**
   3) Visible solid materials in serum samples should be separated by centrifugation.
   4) Prepare the 1 ㎖ deep-well-plate (DWP, 96-well, not offered) or suitable tubes.
   5) Dilute test samples 1/50 with dilution buffer (③). (e.g., by diluting 10㎕ of sample with 490㎕ of dilution buffer(③)). Diluted Samples should be mixed prior to dispensing into the Antigen Coated Plate(①).
4. **Do not dilute the Positive Control(PC,⑤) and Negative Control (NC,⑥)**
5. TMB Substrate (⑦) should be warmed up for 30 minutes at room temperature (20~25℃) before use (10㎖/plate). If stored at low temperature, the color development may be poor.

TEST PROCEDURE

1. **Remove the Antigen Coated Plate (①) from protective foil pouch.**
2. Dispense 100㎕ of diluted samples into appropriate wells. **Use care not to spill samples from well to well.**
3. Dispense 100㎕ of undiluted NC(⑥) and undiluted PC(⑤) into triplicate wells.
4. Seal the plate and incubate for 30 minutes at room temperature (20~25℃).
5. Wash each well 3 times with 1X washing buffer (300㎕ per well). And remove contents of well at each washing steps. After final washing, hit plate to the paper towel briefly to remove solution.
7. Dispense 100㎕ of HRPO Anti-Bovine IgG Conjugate (④) to each well.
8. Seal the plate and incubate for 30 minutes at room temperature (20~25℃).
9. Wash each well as step 5.
10. Dispense 100㎕ of TMB substrate (⑦) to each well.
11. Seal the plate and incubate for 10 minutes at room temperature (20~25℃). Check the density of color development by naked eyes.
12. Add 50㎕ of Stop Solution (⑧) to each well of the plate. Shake the test plate by gently tapping shortly (5~10 sec.). **Be careful not to spill.**
13. Measure and record the A (450nm) for samples and controls immediately.
14. Validate and calculate the results.

Plate template example (1-well Test)

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	13	21	29	37	45	53	61	69	77	85
B	NC	NC	14	22	30	38	46	54	62	70	78	86
C	1	7	15	23	31	39	47	55	63	71	79	87
D	2	8	16	24	32	40	48	56	64	72	80	88
E	3	9	17	25	33	41	49	57	65	73	81	89
F	4	10	18	26	34	42	50	58	66	74	82	90
G	5	11	19	27	35	43	51	59	67	75	83	91
H	6	12	20	28	36	44	52	60	68	76	84	92

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RESULT INTERPRETATION

1. Validate if the mean OD of the PC is higher than 0.50 and the mean OD of the NC is lower than 0.20. If these criteria are not met, the test are invalid and the samples must be retested.
2. Calculate the sample to positive ratio (S/P ratio) by following the formula

$$S/P = \frac{\text{(OD of sample –OD mean of NC)}}{\text{(OD mean of PC – OD mean of NC)}}$$

3. Result interpretation
   1) Test samples having ≥ 0.25 S/P are positive.
   2) Test samples having < 0.25 S/P are negative.

S/P value	Interpretation
S/P ≥ 0.25	Positive
S/P < 0.25	Negative

4. Example of result calculation and interpretation
   1) ODs of NC : 0.085, 0.091
      Mean OD = (0.085 + 0.091) / 2 = 0.088 (valid)
   2) ODs of PC :1.121, 1.201
      Mean OD = (1.121 + 1.201) / 2 = 1.161 (valid)
   3) OD of Sample : 0.431
      S/P value of the sample
      = (0.431 – 0.088)/(1.161 – 0.088) =0.3197
   4) Result interpretation: Positive

PRECAUTIONS

1. All reagents must be allowed to come to room temperature (20~25℃) before use. Mix reagents by gentle swirling. After use, return to 2~8℃.
2. Read this instruction manual thoroughly and follow all steps strictly for successful use of the product.
3. All test samples should be considered potentially infectious and all items contacting the samples should be considered contaminated.
4. Do not use expired or contaminated reagents.
5. Do not use reagents from other kits or lots.
6. Do not mix reagents from different lots of this same product.
7. Do not expose the reagents to excessive heat or direct light during storage and incubation.
8. Incomplete washing adversely may affect the result and precision of the assay.
9. Avoid microbial contamination of the reagents.
10. Avoid contamination of the TMB Substrate(⑦) with the HRPO Anti-Bovine IgG Conjugate(④).
11. Wear personal protective equipment (PPE) such as lab coat, goggle, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
12. Do not eat, drink, smoke or apply cosmetics where kit reagents are handled. Do not pipette by mouth.
13. Pipette tips must be changed after each pipetting step. Use a clean disposable pipette tip for all steps.
14. Use care not to spill samples from well to well.
15. Deionized water or equal must be used to prepare the washing buffer.
16. Unused strips should be stored in the sealed foil pouch at 2~8℃. Re-sealed strips are recommended to use within one week.
17. For veterinary use only.

STORAGE AND STABILITY

Store all reagents at 2~8℃. Do not freeze. Reagents remain stable until the expiration date when stored as instructed.

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QUICK PROTOCOL

Serum dilution 1/50

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Brucella Antigen Coated Plate

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Add 100㎕ of diluted Samples
&
Add 100㎕ of Undiluted PC, NC

RT, 30min → Washing 3 times

Dispense 100㎕ of
HRPO Anti-Bovine IgG Conjugate

RT, 30min → Washing 3 times

Dispense 100㎕ of
TMB Substrate

RT, 10min

Dispense 50㎕ of
Stop Solution

↓

Measure OD at 450nm