Bovine Ephemeral Fever Virus Antibody ELISA Kit

Catalog No: E-AD-E079

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit is comprised by ELISA Microtiter plate pre-coated with Bovine Ephemeral Fever Virus antigen, HRP conjugate and other auxiliary reagents, and apply the principle of enzyme-linked immunoassay (ELISA) to detect bovine BEFV antibody of bovine serum, plasma samples. During the experiment, add control serum, samples and HRP conjugate into the ELISA Microtiter plate. If BEFV antibody exist in the samples, it will be bound with the protein on the microtiter plate after incubation. Then wash the plate to remove unbound antibody and other components. Substrate reagent is added into the well, it will react with the enzyme and the products become blue. The color shade is of positive correlation with antibody levels in the samples. At last, end the reaction by adding stop solution to produce a yellow product. Measure the absorbance value of each well by using a microplate Reader with 450 nm wavelength, then we can know whether there are bovine BEFV antibody in the samples.

Kit components

Item	Specification
ELISA Microtiter plate	96 wells
HRP Conjugate	10 mL
Sample Diluent	6 mL
20×Concentrated Wash Buffer	25 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Positive Control	0.5 mL
Negative Control	0.5 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Experimental instrument

Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)

High-precision transferpettor, EP tubes and disposable pipette tips

37℃ incubator or water bath

Deionized water

Absorbent paper

Notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. All the waste should be handled as contaminant.
2. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contacted carelessly.
3. The ELISA plate obtained from cold storage conditions should be adjusted to room temperature before opening the bag. The unused plate should be kept in a sealed bag with desiccant.
4. Concentrated wash buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should keep fresh.
7. The results shall depend on the readings of the Microplate Reader.
8. **Each reagent is optimized for use in the E-AD-E079. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E079 with different lot numbers.**
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Storage and expiry date

Store at 2~8℃. Avoid freeze.

Please store the opened plate at 2~8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample and reagents preparation

1. **Serum/Plasma:** Use the conventional method to prepare animal serum or plasma, the samples must be clear, no hemolysis and no pollution .Samples can be stored at 2~8℃ for 1 week and at - 20℃ for a long term storage. If the precipitates appear during reservation, the sample should be centrifuged.
2. **Wash Buffer:** Adjust the **20×Concentrated Wash Buffer** to room temperature before use, shake it and make it dissolve fully if appearing salt crystals, then dilute it with distilled or deionized water at 1:19.

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8℃.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 2 wells for negative/positive control respectively. **Samples need test in duplicate.**
2. **Add sample:** add 50 μL of **positive/negative control** to positive/negative control well, and add 10 μL of sample and 40 μL of **Sample Diluent** to the sample wells. Add 100 μL of **HRP conjugate** into each well.
3. **Incubate**: cover the plate sealer and mix thoroughly, and incubate at 37℃ for 60 min in shading light.
4. **Wash:** remove the liquid in each well. Immediately add 350 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 5 times, 60s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **Color Development:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** into each well and mix thoroughly. Cover the plate sealer and incubate at 37℃ for 15 min in shading light.
6. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
7. **OD Measurement:** measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm wavelength (use 630 nm as reference wavelength). This step should be finished in 15 min after stop reaction.

Reference value

Normally, the A-value of negative control ⩽ 0.15 and A-value of positive control ⩾ 1.00 .

Analysis of results

1. Calculate the Cut Off: (C.0) = average A value of negative control (NC) +0.15
2. Positive result: A value of average sample ⩾ Cut Off
3. Negative result: A value of average sample ＜ Cut Off
4. If A value of average sample= Cut Off, please repeat the sample test,

Limitations of this test method

1. This test is only used for the qualitative detection of EFV-Ab in samples of Bovine.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.