Technical Manual

Cattle Hydatidosis Antibodies ELISA Kit

- **Catalogue Code: ADES0043**
- **Antibody ELISA Kit**
- **Research Use Only**

1. Test principle

This kit is comprised by HRP conjugate, other reagents and ELISA Microtiter plate pre-coated with Hydatidosis (HD) antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect HD-Ab in serum of canine. During the experiment, add control and samples into the ELISA Microtiter plate. If HD-Ab exist in the samples, it will be bound with the antigen on the ELISA Microtiter plate. Then wash the plate to remove unbound components, add the HRP conjugate to specifically bind with the compound of antibody and antigen on the microtiter plate. The unbound HRP Conjugate will be removed by washing. Substrate Reagent is added into the well, it will react with the enzyme and become blue. The color shade is positive correlation with HD-Ab 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 judge whether HD-Ab exist in the sample.

2. Kit components

Item	Specification
ELISA Microtiter plate	96 wells
HRP Conjugate	11 mL
Sample Diluent	50 mL
20×Concentrated Wash Buffer	40 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Positive Control	1 mL
Negative Control	1 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.

3. Notes

- 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.
- The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contacted carelessly.
- FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.

- 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.
- Each well must be filled with liquid when washing in order to prevent residual free enzyme.
- The tested sample should keep fresh.
- The results shall depend on the readings of the Microplate Reader.
- **Each reagent is optimized for use in the ADES0043. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ADES0043 with different lot numbers.**
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

4. Storage and expiry date

Store at 2-8° C. Avoid freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.
Expiry date: expiration date is on the packing box.

5. 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° C incubator or water bath
Deionized water
Absorbent paper

6. Sample and reagents preparation

1. **Serum:** Use the conventional method to prepare animal serum, the samples must be clear, no hemolysis and no pollution .Samples can be stored at 2-8° C for 1 week and at -20° C for a long term storage.
2. **Diluted serum**: Dilute the sample serum with the **Sample Diluent** at 1:9 (10 µL sample serum and 90 µL of sample diluent, mix fully). The positive/negative control do not need to be diluted.
3. **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 deionized water at 1:19.

7. Assay procedure

Restore all reagents and samples to room temperature (25° C) 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° C.

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 100 μL of **positive/negative control** to positive/negative control well, and add 10 μL of **Diluted serum** and 90 μL of **Sample Diluent** to the sample wells.
3. **Incubate**: cover the plate sealer and mix thoroughly, and incubate at 37° C for 30 min in shading light.
4. **Wash:** remove the liquid in each well. Immediately add 300 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 5 times, 30 s 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. **HRP Conjugate:** add 100 μL of **HRP Conjugate** into each well, cover the plate sealer and incubate at 37° C for 30 min in shading light.
6. **Wash:** repeat step 4 for washing.
7. **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° C for 10 min in shading light.
8. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
9. **OD Measurement:** measure the absorbance value (A-value) of each well by using a micro-plate reader with 450 nm wavelength (use 630 nm as reference wavelength). **Note: Read the results within 10 min.**

8. Reference value

Normally, the A-value of negative control ≤ 0.15 and A-value of positive control ≥ 0.6.

9. Interpretation of the results

1. Positive result: A-value of sample ≥ 0.5
2. Negative result: A-value of sample < 0.5

10. Limitations of this test method

1. This test is only used for the qualitative detection of HD-Ab in serum of canine.
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.

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