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Optimize Your Research

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**Qualitative ELISA Kit User Instruction**

# Horse Babesia caballi Antibody ELISA Kit

## Cat.NO BTB-ED0005HO

***This product is for research use only, not for use in diagnosis procedures. It's highly recommend to read this instruction entirely before the use.***

**Storage**: Store the reagents at 2-8°C. For long term storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

## Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.
Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.
CV(%) = SD/mean x 100
Intra-Assay: CV< 10%
Inter-Assay: CV< 12%

## Intended Use

This kit is for the qualitative detection of Horse Babesia caballi Antibody in serum, plasma, cell culture supernates, cell lysates, tissue homogenates or other biological fluids..

## Assay Principle

This kit is based on a qualitative reverse phase enzyme immuno assay technique. The microtiter plate has been pre-coated with a target antigen. Positive/Negative Controls or samples are added to the wells and incubate. Antibodies in the samples bind to the antigen on the plate. Unbound antibody is washed away during a washing step. A Horseradish Peroxidase (HRP) conjugated detection antibody is then added and incubate. Unbound HRP is washed away during a washing step. TMB substrate is then added and color develops. The reaction is stopped by addition of acidic stop solution and color changes into yellow that can be measured at 450 nm. The OD of an

unknown sample can then be compared to the OD of the positive and negative controls in order to determine the presence of Babesia caballi Antibody.

## Reagents Provided

| Components | Quantity |
|---|---|
| Pre-coated Plate | 12 * 8 well strips x 1 |
| Positive Control | 0.5ml × 1 vial |
| Negative Control | 0.5ml × 1 vial |
| HRP Conjugated | 6ml × 1 vial |
| Sample Diluent | 6ml × 1 vial |
| Substrate Solution A | 6ml ×1 vial |
| Substrate Solution B | 6ml ×1 vial |
| Stop Solution | 6ml ×1 vial |
| Wash Buffer (25x) | 20ml × 1 vial |
| Plate Sealer | 2 pcs |
| Zipper Bag | 1 |
| User Instruction | 1 |

## Materials Required But Not Supplied

- 37°C incubator
- Precision pipette and disposable tip
- Deionized or distilled water
- Clean tube
- Absorbent paper
- Automatic plate washer or 8-channel pipette
- 500ml beaker and suitable measuring cylinder

## Precautions

- Prior to running the assay, the kit and sample should be warmed naturally to room temperature 30 minutes.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
- Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
- Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
- Do not allow wells to become dry during the assay procedure.

- Avoid using the reagents from different batches together.
- Substrate B is sensitive to light, don't expose substrate solution B to light for a long time.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
- The kit should not be used beyond the expiration date.

## Specimen Collection

**Serum** Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

**Plasma** Collect plasma using EDTA or heparin as an anticoagulant.After mix 10-20 minutes, centrifuge samples for 20 minutes at 2000-3000 RPM. Collect the supernatant without sediment.

**Urine/Ascites/ Cerebrospinal fluid** Collect by sterile tube. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

**Cell culture supernatant** Collect by sterile tubes. When detecting secrete components, centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatants. When detecting the components in the cell, use PBS (pH 7.2-7.4) to dilute cell suspension , the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

**Tissue** Rinse tissues in ice-cold PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to get the supernatant.

## Note

- The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the sample used in the whole test.
- Samples to be used within 5 days should be stored at 2-8°C. Samples should be aliquoted or must be stored at -20°C within 1 month or -80°C within 6 months. Avoid repeated freeze thaw cycles.
- Samples should be brought to room temperature before starting the assay.
- Samples containing NaN3 can't be tested as it inhibits the activity of HRP.
- Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
- Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

## Reagents Preparation

- All reagents should be brought to room temperature before use.
- **Wash Buffer 25x** Dilute 25x wash buffer with distilled water to yield 500 ml of 1x wash buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1: 25 with reagent grade water.

## Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 4°C for up to one month.
3. Set a Blank well without any solution.
4. Add 50μl negative control to each of the negative control wells and 50μl positive control to each of the positive control wells. Add 40μl sample diluent and then add 10μl sample to the sample well, mix well.
5. Cover with a plate sealer, and incubate for 30 minutes at 37°C.
6. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
7. Add 50μl HRP to each well (except blank well) .Cover with a plate sealer, and incubate for 30 minutes at 37°C.
8. Remove the sealer and wash as described above.
9. Add 50μl substrate solution A to each well and then add 50μl substrate solution B to each well.Mix well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
10. Add 50μl stop solution to each well, the blue color will change into yellow immediately.
11. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 15 minutes after adding the stop solution.

## Summary

Prepare all reagents, samples and controls and set a blank control with no solution.

Add 50µl positive control or negative control to each well.C

Dilute 10µl sample with 40µl sample diluent and add this diluted sample to each well.

Incubate for 30 minutes at 37°C.

Aspirate and wash 5 times.

Add HRP to each well and incubate for 30 minutes at 37°C

Aspirate and wash 5 times.

Add substrate solution A and substrate solution B and incubate in the dark for 10 minutes at 37°C.

Add 50µl stop solution.

Read the OD value within 15 minutes at 450nm.

## Calculation of Results

Average the reading from duplicate or triplicate samples.

For calculation the valence of Horse Babesia caballi Antibody, compare the sample well with control.

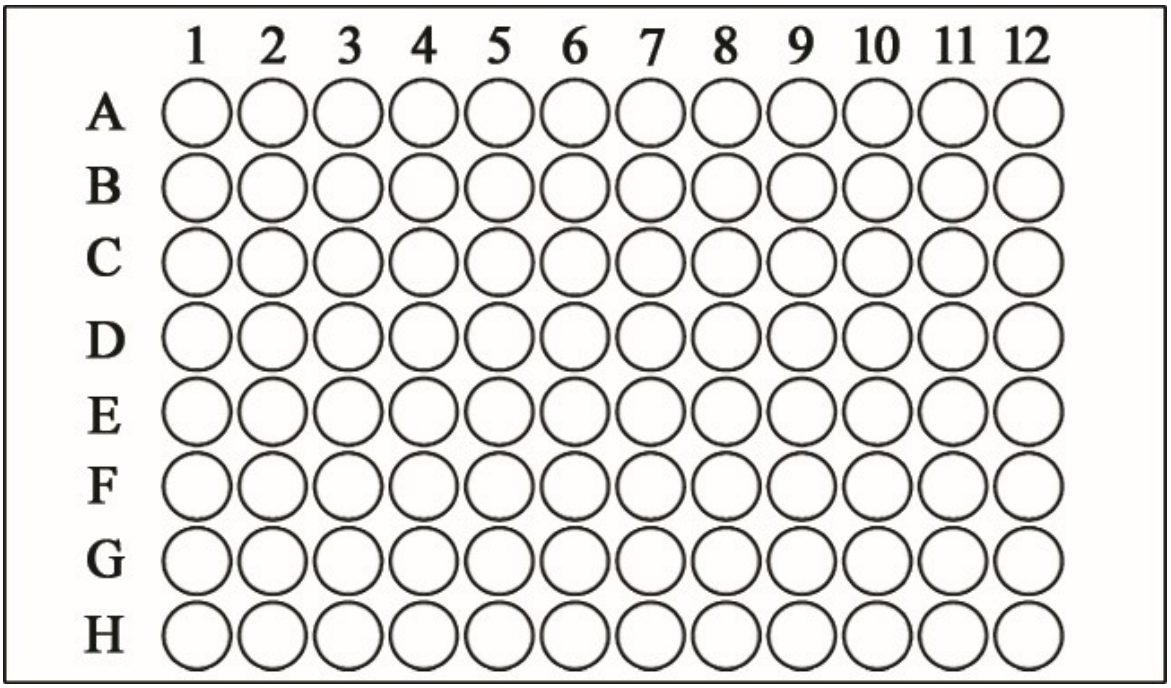
**Quality Control**

- The average $OD_{positive}$ ≥1.00
- The average $OD_{negative}$ ≤0.10

**Results**

- Cutoff Value = average Negative Control value + 0.15
- While $OD_{sample}$ < Cutoff Value: Negative ·
- While $OD_{sample}$ ≥Cutoff Value: Positive

## Troubleshooting

| Possible Case | Solution |
|---|---|
| **High Background** | |
| • Improper washing | • Increasing duration of soaking steps |
| • Substrate was contaminated | • Replace. Substrate should be clean and avoid crossed contamination by using the sealer |
| • Non-specific binding of antibody | • Replace another purified antibody or blocking buffer |
| • Plate are not be sealing incompletely | • Make sure to follow the instruction strictly |
| • Incorrect incubation temperature | • Incubate at room temperature |
| • Substrate exposed to light prior to use | • Keep substrate in a dark place |
| • Contaminated wash buffer | • Use a clean buffers and sterile filter |
| **Weak Signal** | |
| • Improper washing | • Increasing duration of soaking steps |
| • Incorrect incubation temperature | • Incubate at room temperature |
| • Antibody are not enough | • Increase the concentration of the antibody |
| • Reagent are contaminated | • Use new one |
| • Pipette are not clean | • Pipette should be clean |
| **No Signal** | |
| • Reagent are contaminated | • Use new one |
| • Sample prepared incorrectly | • Make sure the sample workable/dilution |
| • Antibody are not enough | • Increase the antibody concentration |
| • Wash buffer contains sodium azide | • Use a new wash buffer and avoid sodium azide in it |
| • HRP was not added | • Add HRP according to the instruction |
| **Poor Precision** | |
| • Imprecise/ inaccurate pipetting | • Check/ calibrate pipettes |
| • Incomplete washing of the wells | Make sure wells are washed adequately by filling the wells with wash buffer and all residual antibody solutions crossed well before washing. |

**Data Template**