Infectious Bronchitis Virus Antibodies ELISA Kit
Catalog No: E-AD-E012
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) 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

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 Micro ELISA Plate pre-coated with Infectious Bronchitis Virus (IBV) recombinant N protein, HRP conjugate and other auxiliary reagents and apply the principle of enzyme-linked immunoassay (ELISA) to detect IBV antibody in samples. During the experiment, add control serum and samples into plate. If AIV antibodies exist in the samples, it will be bound with the antigen pre-coated to the micro-plate. Then wash to remove unbound antibodies and other components, add the HRP conjugate to specifically bind with the compound of antibody and antigen on the micro-plate and the unbound HRP conjugate will be removed by washing. Add TMB substrate in the wells, it will react with the enzyme and become blue, the shade of color is of positive correlation with IBV 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 micro-plate reader with 450 nm wavelength, then we can judge whether IBV antibody exist in the sample.

Kit components

<table>
<thead>
<tr>
<th>Item</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro ELISA Plate</td>
<td>96 wells</td>
</tr>
<tr>
<td>HRP Conjugate (red cap)</td>
<td>11 mL</td>
</tr>
<tr>
<td>Sample Diluent (yellow cap)</td>
<td>50 mL</td>
</tr>
<tr>
<td>20×Concentrated Wash Buffer</td>
<td>40 mL</td>
</tr>
<tr>
<td>Substrate Reagent A (white cap)</td>
<td>6 mL</td>
</tr>
<tr>
<td>Substrate Reagent B (black cap)</td>
<td>6 mL</td>
</tr>
<tr>
<td>Stop Solution (yellow cap)</td>
<td>6 mL</td>
</tr>
<tr>
<td>Positive Control (red cap)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Negative Control (green cap)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3 pieces</td>
</tr>
<tr>
<td>Sealed Bag</td>
<td>1 piece</td>
</tr>
<tr>
<td>Manual</td>
<td>1 copy</td>
</tr>
</tbody>
</table>

Experimental instrument
Micro-plate 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 or distilled water
Absorbent paper
Sample preparation
1. Use the conventional method to prepare animal whole blood serum, the serum must be clear, no hemolysis and no pollution. Samples can be conserved in 2~8°C in 1 week, and it should be stored at -20°C for a long term storage.
2. Dilute the sample serum with the sample diluent at 1:99 (2 μL of sample serum and 198 μL of sample diluent, mix properly). The positive/negative control do not need to be diluted.
3. The 20×Concentrated wash buffer should be adjusted to room temperature before used, then dilute it with deionized or distilled water at 1:19.
4. Bring all reagents to room temperature (18~25°C) for 30 min before use.

Assay procedure
1. Take out the micro-plate, set 1 well for blank control and 2 wells for negative/positive control respectively. The unused Micro ELISA Plate should be sealed as soon as possible and stored at 2~8°C.
2. Add 100 μL of sample diluent solution to the blank control well, add 100 μL of positive/negative control to positive/negative control wells. Add 100 μL of diluted sample to the sample wells.
3. Gently tap the plate to ensure thorough mixing, incubate at 37°C for 30 min.
4. Aspirate each well and wash, repeat the process 5 times, immerse 30-60 sec each time. Wash by filling each well with wash buffer (approximately 350 μL) (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). Complete removal of liquid at each step is essential. After the last wash, remove remained wash buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.
5. Add 100 μL of HRP conjugate into each well (except the blank control well), and incubate at 37°C for 30 min.
6. Repeat step 4 for washing.
7. Add 50 μL of substrate reagent A and 50 μL of substrate reagent B into each well, gently tap the plate to ensure thorough mixing, incubate for 10 min at 37°C. Protect from light.
8. Add 50 μL of stop solution into each well, gently mix. Adjusted zero with the blank control, measure the absorbance value (A-value) of each well by using a micro-plate reader in 450 nm wavelength (use 630 nm as reference wavelength).
Reference value
Normally, the OD of negative control $\leq 0.1$ and the OD of positive control $\geq 0.6$.

Interpretation of the results
1. Cut Off (C.O) = 0.13 + average of negative ODs (Take it as 0.07 if the average of negative ODs $< 0.07$.)
2. Positive result: OD $\geq$ C.O;
   Negative result: OD $< C.O.$

The negative result of this test suggests that the concentration of antibody for the tested sample is not enough, it is recommended that this animal should get immunized with corresponding vaccine.

Limitations of this test method
This test is only used for the qualitative detection of IBV-IgG antibody in chicken serum and plasma. A rough estimate (high, general, low) of the concentration of this antibody can be concluded according to the OD values.

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 micro-plate reader.
8. Do not use components from different batches of kit.

Storage and Expiry date
Store at 2~8°C with shading light for 12 months.
Please store the opened plate at 2~8°C, protect from light and moisture. The valid period is 2 months.