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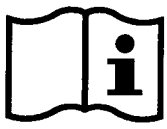

Useŕs Manual

Infectious Bovine Rhinotracheitis Ab ELISA

Enzyme Immunoassay for the detection antibodies against the antigen of BHV type 1 in bovine serum and plasma

Kat. / Cat #: **DE3160**
Tests: **96 wells**

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1 INTRODUCTION

Infectious Bovine Rhinotracheitis (IBR) is a severe respiratory herpes virus infection in cattle characterized by tracheitis, rhinitis and fever. IBR is transmitted horizontally by contact with respiratory, ocular and reproductive secretions. IBR also acts as an immunosuppressive, predisposing individuals to secondary bacterial infections. Despite eradication programs for IBR in many parts of the world, infection with IBR remains endemic in many cattle populations resulting in serious economic losses.

Serological identification of IBR infected cattle is routinely performed by screening serum samples for antibodies, using serum neutralization tests and indirect ELISAs. These tests suffer some disadvantages. They are time consuming, insensitive and difficult to read. To detect antibodies in milk samples, more sensitive test systems are required.

This ELISA is intended to use as a rapid screening test for the detection of anti-IBR antibodies in serum and plasma samples of infected cattle.

2 INTENDED USE

This diagnostic test is intended to identify antibodies against the antigen of BHV-1 virus in serum and plasma samples.

3 PRINCIPLE

The test is based on the reaction of anti-I BR antibodies with BHV-1 antigen. To this end these BHV-1 antigens are coated to a 96-well microtiter strip plate.

The bovine serum or plasma sample is added to the wells of the coated plate.

After incubation, a anti-BHV-1 conjugate is added to compete for the BHV-1 antigen that is coated to the 96-well microtiter strip-plate.

Bound conjugate is made visible by adding substrate/chromogen mix.

Intensity of the colour reaction in the wells is directly correlated to the concentration of anti-IBR antibodies in the serum, plasma or milk sample.

4 CONTENTS

- 12 x 8 **microtiter strips**
- 1 x **strip holder**
- 1 x 8 ml **ELISA buffer**
- 1 x 12 ml **HRPO-conjugated anti-BHV-1 antibodies**
- 1 x 1 ml **Positive Control**
- 1 x 1 ml **Negative Control**
- 1 x 60 ml **Wash Solution 200 x** concentrated (Dilute in deionized water before use!)
- 1 x 8 ml **Substrate Buffer A**
- 1 x 8 ml **Substrate Buffer B**
- 1 x 8 ml **Stop Solution**.
- 1 x Plastic cover seal

5 HANDLING AND STORAGE OF SPECIMENS

The ELISA should be stored at 4-8°C.

An unopened package can be used until the expiry date.

An opened package can be used if the requirements, mentioned in the validation (See 9.), are fulfilled, if not fulfilled the test can no longer be used.

Avoid repeated cooling and thawing as this increases non-specific reactivity.

Samples may be used fresh or may be kept frozen below -20°C before use.

Positive and negative controls may be stored in aliquots at -20°C and used until the expiry date.

6 WASH PROTOCOL

In ELISA's, uncomplexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better results.

Manual washing

1. The wash-solution is 200x concentrated**, dilute it in de-ionized water before use!**
2. Empty each well by turning the microtitre plate upside down, followed by a firm vertical downward movement to remove the contents of the wells.
3. Fill all the wells with 250 µl washing solution.
4. This washing cycle (2 and 3) should be carried out **at least 4 times**.
5. Turn the plate upside down and empty the wells by a firm vertical downward movement.
6. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove residual washing solution in the wells.
7. Take care that none of the wells dries out before the next reagent is dispensed.

Washing with automatic equipment

The wash-solution is 200x concentrated**, dilute it in de-ionized water before use!**

When using automatic plate washing equipment, check that all wells can be aspirated completely and that the washing solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute **at least 4 washing cycles**.

7 PRECAUTIONS

- Handle all biological material as though capable of transmitting CPV.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.
- Do not use components past the expire date and do not mix components from different serial lots together.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect is from damage and dirt.

8 TEST PROTOCOL

1. Open the packet of strips and take out the strips to be used. One well for each sample.
   Leave the remaining strips covered in the plastic and store at 4-8°C.
2. **Serum/plasma samples:**
   Add 50 µl of ELISA buffer to all wells to be used. To one well of the coated strip add 50 µl of positive control and to another well 50 µl of negative control.
   In addition, add 50 µl serum/plasma of each sample to an individual marked sample well of the strip.
3. Seal and incubate for 3 hours at 37ºC
4. Wash the microtiter strip(s) with washing solution, according to washing protocol.
   The washing solution provided has to be diluted 2000 x in de-ionized water!
5. Dispense 100 µl of conjugate to all wells.
6. Seal and incubate 30 minutes at room temperature
7. Wash as in 4.
8. Mix equal parts of Substrate A and Substrate B with gentle shaking. Prepare immediately before use!
9. Dispense 100 µl substrate solution to each well.
   Incubate for 15 min. at room temperature (21ºC).
10. Add 50 µl stop solution to each well (mix well).
11. Read the absorbency values immediately (within 10 min.!) at 450 nm. Use 620nm as a reference.

9 VALIDATION OF THE TEST

To standardize the Bovine Rhinotracheitis Virus ELISA, positive and negative controls have to be tested.

10 INTERPRETATION OF TEST RESULTS

Calculation:

Ratio = Sample OD/Negative control OD

A sample is considered positive when the ratio is ≤ 0,6 ,

negative when the ratio is ≥ 0,7 and the sample should be retested when between 0,6 and 0,7.

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