DRG® IBR 3 step HS ELISA (EIA-3160)

For Veterinary Use Only

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. Screening serum samples for antibodies, using serum neutralization tests and indirect ELISAs routinely performs serological identification of IBR infected cattle. 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 blocking based ELISA is intended to use as a rapid screening test for the detection of anti-IBR antibodies in serum and milk samples of infected cattle.

INTENDED USE OF THE IBR TEST KIT

This diagnostic test is intended to identify antibodies against several epitopes of IBR virus, in serum and milk samples. In contrast to test systems, which make use of neutralizing antibodies, this blocking based, ELISA has a very high sensitivity and blocking specificity. Dilution of serum or milk samples is not required, but a 1:2 dilution of the serum samples is recommended to obtain more reproducible results.

STANDARDIZATION

To standardize the IBR ELISA, positive and negative standards are tested which resulted in a new IBR ELISA standard. The negative control should give an OD of approximately 0.900 and the (weak) positive control should give an OD of approximately 0.500.

PRINCIPLE OF THE IBR TEST

Undiluted milk of serum samples are added to the pre-coated wells. After incubation and appropriate washing, a polyclonal anti-IBR antibody conjugated to Biotin is added and the plates are again incubated. After appropriate washing, a streptavidin peroxidase conjugate is added and the plates are again incubated. After washing, substrate is added. After 15-25 minutes, the color reaction is stopped and the plates are immediately read at 450 nm.

CONTENTS OF THE IBR TEST KIT

- 2 x 96 well microtiter plates coated with IBR antigen
- 2 x 10 ml biotin-conjugated (anti-IBR) polyclonal antibody (freeze-dried).
- 2 x 10 ml HRPO-conjugated streptavidin (freeze-dried)
- 1 x 1 ml inactivated positive control (freeze-dried)
- 1 x 1 ml inactivated negative control (freeze-dried)
- 1 x 60 ml wash solution 200x concentrated (Dilute in deionized water before use)
- 2 x 10 ml ELISA buffer
- 1 x 11 ml substrate buffer A
- 1 x 11 ml substrate buffer B
- 1 x 11 ml stop solution
- 1 x 100 ml diluent

STORAGE OF KITS

The kit should be stored at 2-8°C. After reconstitution, the lyophilized reagents should be used immediately.

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HANDLING AND STORAGE OF SPECIMENS

Fresh samples can be used without restriction. Addition of 0.1% sodium azide to the samples has no influence on the results. For prolonged storage, samples should be frozen as soon as possible and stored at –25°C until use. Avoid repeated freezing and thawing as this increases non-specific reactivity and decreases the end-point titer.

- **Milk samples—undiluted**
  For optimal sensitivity, pooled milk samples can be tested undiluted. To avoid false positive reactions, defatted samples must be used. Centrifuge the milk samples for 15 minutes at 2500 g and take a sample from below the fat layer.

- **Serum samples**
  It is preferable that individual serum samples be diluted 1:2 in ELISA buffer, but can also be tested undiluted.

WASHING PROTOCOL

In ELISA's between each immunological incubation step up, complexed components have to be removed efficiently. 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 advised to carefully follow the washing procedures outlined below. Both manuals washing and washing with automatic equipment can be performed. (Automatic washing equipment usually gives better results).

Manual Washing

1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical movement.
2. Fill all the wells with 250 µl washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 times.
4. Turn the plate upside down and empty the wells by a firm short vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove residual washing solution in the wells.
6. Take care that none of the wells dries out before the next reagent is dispensed.

Washing with automatic equipment

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.

TEST PROTOCOL

1. Wash the microtiter plate with washing solution, according to washing protocol.
   The washing solution provided has to be diluted 10x.
2. Reconstitute the positive control in 1 ml deionized water.
   Reconstitute the negative control in 1 ml deionized water.
   Reconstitute ELISA buffer in 10 ml diluent.
   Add 150µl positive control into wells A1 and B1, 150 µl negative control into wells C1 and D1 and 150 µl ELISA buffer into wells E1 and F1 of a round-bottomed plate.

3. Serum (150µl): Diluted (1:2 in ELISA buffer) or undiluted serum samples are added to the other wells of the round bottom plate. Milk (150µl): For milk samples, add 100µl undiluted milk to the wells of the round-bottomed plate.
4. Transfer 100µl of controls and samples to the coated microtiter plate.
5. Seal the microtiter plate and incubate for 60 min. at 37°C.
6. Wash as 1.
7. Reconstitute the biotin-conjugated antibody directly before use with 10 ml diluent. Immediately dispense 100µl of this conjugate to all wells.
8. Seal and incubate 60 minutes at 37°C.
9. Wash as 1.
10. Reconstitute the HRPO-conjugated streptavidine directly before use in 10 ml diluent. Immediately dispense 100µl of this conjugate to all wells.
11. Seal and incubate 30 minutes at 37°C.
12. Mix equal parts of buffer A and buffer B together with gentle shaking. Prepare immediately before use! Dispense 100µl substrate solution to each well. Incubate 15-25 min. at room temperature (21°C).
13. Add 50µl stop solution to each well (mix well).
14. Read the absorbency values immediately (within 10 min) at 450nm. Use as a reference wavelength 620nm.

PRECAUTIONS

- Handle all biological materials as though capable of transmitting IBR
- 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 if swallowed; observe care when handling the substrate.
- Do not use components past their expiry date and do not intermix components from different serial lots.
- 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 microtitire plate and prevent it from damage and dirt.

VALIDATION OF THE TEST

In order to confirm appropriate test conditions the mean OD of the negative control should be approximately 0.900 (450nm). The IBR (weak) positive ELISA standard provided should give an OD of approximately 0.500 (450nm).

INTERPRETATION OF TEST RESULTS

In general high prevalence when more than 15% is positive. This prevalence can be used for a certain area (f.i. a farm, state, or country) depending on elimination campaigns or other (government) restrictions.

Low prevalence

A sample is scored negative if the OD value is > 80% of the OD of the negative control. A sample with an OD value between 60% and 80% of the OD of the negative control is considered weak positive or doubtful. This sample must be retested after a certain period of time. A sample is scored positive if the OD value is <60% of the OD of the negative control.

High prevalence
A sample is scored negative if the OD value is > 75% of the OD of the negative control.
A sample is scored positive if the OD value is < 75% of the OD of the negative control.