Infectious Bovine Rhinotracheitis gB Antibody ELISA test kit

INSTRUCTION MANUAL

FOR PRODUCT No: LT018001BZ5

Arsh Biotech Pvt. Ltd.
308, Aggarwal City Mall, Road No.44,
Pitampura, Delhi-110034, India
Mobile: +91-98105-21400 | Fax: +91-11-42208444
info@arshbiotech.com

MANUAL VERSION 1.01

INTRODUCTION

Infectious Bovine Rhinotracheitis gB (IBR) antibody Elisa test kit can be used to detect Infectious Bovine Rhinotracheitis gB antibody in serum or plasma of Bovine.

PRINCIPLE

This kit use indirect ELISA method, pured IBR antigen is pre-coated on micro-well strips. When testing, add diluted serum sample, after incubation, if there is IBR specific antibody, it will combine with the pre-coated antigen, discard the uncombined antibody and other components by washing, then add enzyme conjugate, discard the uncombined enzyme conjugate by washing. Add TMB substrate in micro-wells, the blue signal by Enzyme catalysis is directly proportion of antibody content in sample.

MATERIASL SUPPLIED WITH THIS KIT

1	Antigen coated microplate	96T×5	7	Stop solution	15 ml×5
2	Enzyme conjugate	11 ml×5	8	Washing solution 10X	100 ml×5
3	Sample diluent	100 ml×5	9		10 pieces
4	Negative control	2 ml×5	10	Serum dilution plate	10 pieces
5	Positive control	1.6 ml×5	11	Instruction	1 piece
6	Substrate	11 ml×5			

MATERIALS REQUIRED BUT NOT PROVIDED

1) Micropipette: 0.5µl-10µl,10µl-100µl, 100µl-1000µl.

2) Disposable pipette tips.

3) Cylinder: 500ml. 4) Microplate Reader: 96 wells.

5) Distilled water or deionized water. 6) Microplate Washer

SAMPLE PREPARATION

Take animal whole blood, get serum by using regular method, the serum should bright and no hemolysis

WASHING BUFFER PREPARATION

Return 10X Concentrated washing buffer into room temperature before use, if there is salt crystals, shake to make it dissolved, then dilute it at 10 times with distilled water or deionized water. The diluted washing buffer can store at 4℃ for about 1 month.

DILUTION OF SAMPLE

Dilute the sample to be tested (198µl sample dilution + 2µl sample serum) by 1:100 on the dilution plate. Dilute the milk to be tested (196µl sample dilution + 4µl sample) by 1:50 on the dilution plate.

Note: negative and positive controls need not be diluted. After taking each sample, replace the Micropipette tip and accurately record the position of each sample on the board. Each sample should be thoroughly mixed before adding to the Antigen coated microplate.

PROCEDURE

1) Take the antigen coated microplate (the plate can be open and used for several times according to sample quantity each time), add the diluted serum to reaction wells, 100µl/well; meanwhile, set 2 wells for positive control and 1 well negative control, both positive control and negative control do not need dilute, take 100µl

directly and add into its well, mix gently (do not overflow).

2) Cover with adhesive plate sealer, incubate at 37 ℃ for 30 minutes.

3) Open the adhesive plate sealer, discard the liquid of the well, add diluted washing buffer to each well, 250µl/well, then discard the liquid, repeat the above step for 4-6 times, at last flap to dry with the absorbent paper.

4) Adding Enzyme Conjugate, 100µl/well, cover it with Adhesive plate sealer, incubate at 37 ℃ for 30 minutes.

5) Open the adhesive plate sealer, discard the liquid of the well, washing 4-6 times as step3, remember at last flap to dry with the absorbent paper.

6) Add substrate 100µl/well, mix it evenly then cover it with Adhesive plate sealer, incubate at 37 ℃ in dark for 10 minutes.

7) Add stop solution 50µl/well to stop the reaction, measure the result in 10 minutes.

INTERPRETATION OF RESULTS

Read the OD value with ELISA Reader at 450nm (630nm as reference).

For the assay to be valid:

Negative control (N) OD value< 0.2, meanwhile positive

control (P) OD value > 0.5.

Calculate method:

S/P value = Sample OD Value/Positive control OD average value

Results interpretation

S/P value< 0.3 Negative

S/P value≥0.3 Positive

NOTE

1) Return all reagents into room temperature before use, shake it evenly before use, and store back to 2-8℃ after usage.

2) Do not mix use reagents from different kits and different lot no., prevent the reagents been polluted when using.

3) Substrate and stop solution may have irritation to skin and eyes, be careful to use.

4) Do not expose Substrate to strong light and avoid contact with the oxidant.

5) Pre-coated plates should be sealed and moisture-proof. Put back un-used Microwell plate into dry foil bag and sealed at 2-8 ℃.

6) All wastes should be treated well to avoid pollution before discarding.

7) Strict compliance with the operating instructions can get the best results. Pipetting operation, timing, and washing of the whole process must be precise.

8) Antigen coated plate is disposable, do not re-use.

Specifications: 96 wells ×5.

Expiry date: 12 months.

Storage: at 2-8 ℃, don't expose in strong light.

THIS IS A DRAFT MANUAL FOR REFERENCE PURPOSES ONLY. PLEASE REFER TO MANUAL SUPPLIED WITH KIT FOR EXACT DETAILS.