HANDBOOK

BioLisa® kit IBR gB Ab

ELISA kit for the detection of antibodies to gB protein of BHV-1 virus

Method	Matrix	Species	Protocole
Blocking ELISA	Individual serum	Bovine	Short incubation Long incubation

Cat.No. : 703083

MU/IBRGB/00002/EN

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For veterinary use only
Consistence with the DVC/IBRGB00001EN

• General information

BHV1 is the causative agent of Infectious Bovine Rhinotracheitis (IBR) – a respiratory disease with tracheitis, rhinitis, and fever. Less often, it could also lead to abortions, Infectious Pustular Vulvovaginitis (IPV), and balanoposthitis. As for all HerpesVirus, an infection by BHV-1 may be followed by a latent phase. Reactivation of the virus can be the cause of the infection spreading in the herd.

BioLisa® kit IBR gB Ab is a sensitive and specific ELISA test for the detection of antibodies to gB Protein of BHV-1 virus in bovine individual serum.

• Principle of the test

The plates are coated with an inactivated BHV-1 antigen. During the sample incubation, the anti-BHV-1 antibodies bind to the antigen. After a washing step, an HRP conjugated monoclonal anti-gB antibody is added. This conjugate specifically bind to the gB proteins which remain free on the bottom of the wells. In case of a positive sample, the conjugate cannot bind to the antigen.The unbound conjugate is removed by washing, and the substrat solution is added. By contact with the HRP enzyme, the substrat solution becomes blue. After stopping the reaction, the colour turns to yellow.
The optical density (OD) is measured by a spectrophotometer. The absence of coloration in the well indicate the presence of a positive sample.

• Kit content

	Componant	Quantity
1	Test plate. 12 strips of 8 wells per plate	5
2	Sample diluent	1 x 30mL
3	Negative Control	1 x 3.5mL
4	Positive Control	1 x 3.5mL
5	Wash buffer 10X	3 x 125mL
6	Conjugate	1 x 60mL
7	TMB Substrate	1 x 60mL
8	Stop Solution	1 x 60mL
Certificat of analysis		

The componants must be stored at 5±3°C and are stable until the expiration date indicated on the labels. The wash buffer 10X and the stop solution could be stored at room temperature (18-25°C) to avoid cristalisation. The unused plate strips must be stored in the sealed bag with a desiccate packet at 5±3°C. The test strips can be stored for at least 6 weeks after opening the plate bag.

• Symboles

Contains reagents for <N> plates


Legal manufacturer

Lot number


Use by date


Temperature limitations for storage

Catalog number

Material number


Protect from light


For cattle samples

• Not provided material

- Monochannel and multichannels pipettes
- Single-use tips
- Microplate absorbance reader with a 450nm filter
- Distilled or deionised water
- Single-use containers
- Aluminium or adhesive foil for covering the Test Plate

• General precautions

Always wear appropriate personal protective equipment. For more information, please consult the appropriate material safety data sheets (MSDS) (availables on request).

- Carrefully read and strictly follow the instruction of use
- Handle all materials according to the Good Laboratory Practice.
- Consider all the reagents and samples as potentially contaminated.
- All unused biological materials should be disposed according to the local, regional and national regulations.
- Do not use reagents beyond expiration date.
- Do not mix components from different kit batches.
- Include positive and negative controls on each plate or test strip series.

• Before starting

All reagents should equilibrate to room temperature (21±4°C) before use.

Wash solution : the wash solution 10X must be diluted 10 fold in distilled/ deionised water. *Example : for one plate, dilute 50mL of wash solution 10X into 450mL of water.*
The diluted wash solution could be stored for one month at 5±3°C.

Samples : fresh, refrigerated or frozen individual sera could be used.

• Protocol

1. **Sample dilution 1:2 :**
   Add 50µL of sample diluent into each well.
   Add 50µL of positive control (PC) and negative control (NC) into the appropriated wells *(A1B1 and C1D1 for example).*
   Add 50µL of samples into the following wells.

Gently shake the plate and cover it with an adhesive plate cover. Incubate **2h at 37°C±2°C** (short incubation) or **overnight (12-18h) at room temperature (18-25°C)** (long incubation).

2. **Washing :**
   Empty the wells and **wash 5 times with 300µL** of diluted wash solution. After the last washing step, empty the plate and softly tap it on an absorbant paper to remove all the liquid.

This step could be done manually or with a washing machine.

3. **Conjugate**
   Add 100µL of conjugate in each well.

Cover the plate and incubate **1h at room temperature (18-25°C).**

4. **Washing :**
   Repeat the step **2. Washing**.

5. **Revelation :**
   Add 100µL of substrat in each well.

Incubate the plate **10min at room temperature (18-25°C) in darkness.**

6. **Stopping the reaction :**
   Stop the reaction by adding 100µL of stop solution in each well following the same order than with the substrat.

7. Reading :

Measure the optical density (OD) in a microplate reader at **450nm** within 20min after stopping the reaction. Make sure there is no dust or bubbles into the wells.

• Validation criteria

The results are validated if :

- The mean OD of the negative control is ≥ 0.60.
- The mean % inhibition of the positive control is ≥ 75%.

If one of these criteria is not compliant, the assay should be repeated.

• Calculations

Calculate the mean OD for the positive and negative controls.

The **inhibition percentage** value is calculated for each sample, using the following formula :

$$\% \text{ Inh} = \frac{\text{Mean NC OD} - \text{Sample OD}}{\text{Mean NC OD}} \times 100$$

• Interpretation of results

Short incubation	Long incubation	Interpretation
% Inh < 45%	% Inh < 55%	Negative
45% ≤ % Inh < 55%	55% ≤ % Inh < 65%	Doubtful
% Inh ≥ 55%	% Inh ≥ 65%	Positive

NB : The kit specificity is improved using the long incubation protocol.