BHV1 gB ELISA kit

Prod. No.: DEIA559
Pkg. Size: 5 plates

INTENDED USE

The ELISA kit is a competitive enzyme immunoassay (ELISA) in microtitre plate format for the detection of antibodies to the glycoprotein B of Bovine Herpesvirus 1 (BHV1) in serum, plasma and milk samples from cattle. After sample preparation, pool milk samples can be tested, too.

GENERAL DESCRIPTION

The Bovine Herpesvirus 1 is the causative agent of Infectious Bovine Rhinotracheitis (IBR) – a respiratory disease with tracheitis, rhinitis and fever. In addition BHV1 infections can cause Infectious Pustular Vulvovaginitis (IPV), balanoposthitis and abortions. Clinical disease is often followed by latent BHV1 infection. Reactivation of the virus can be the cause of spreading of the infection in the herd. The BHV1 gB Ab is a competitive ELISA. The Test Plate is coated with inactivated BHV1 antigen. During the sample incubation step, antibodies specific to BHV1 bind to the immobilised antigen; unbound material is removed by rinsing. Afterwards, a HRP-labeled, gB-specific monoclonal antibody is added, which can not bind to the BHV1 antigen while its antigenic determinant is occupied previously by antibodies in the test sample. Unbound anti-gB-HRP conjugate is rinsed out. The colour reaction is started by adding the substrate solution and stopped after 10 minutes. The optical density (OD) is measured in a spectrophotometer. The blocking value (percentage of inhibition) is calculated from the OD values obtained with the test sample and the Negative Control, which contains no BHV1-specific antibodies.

PRINCIPLE OF THE TEST

The BHV1 gB Ab is a competitive ELISA. The Test Plate is coated with inactivated BHV1 antigen. During the sample incubation step, antibodies specific to BHV1 bind to the immobilised antigen; unbound material is removed by rinsing. Afterwards, a HRP-labeled, gB-specific monoclonal antibody is added, which can not bind to the BHV1 antigen while its antigenic determinant is occupied previously by antibodies in the test sample. Unbound anti-gB-HRP conjugate is rinsed out. The colour reaction is started by adding the substrate solution and stopped after 10 minutes. The optical density (OD) is measured in a spectrophotometer. The blocking value (percentage of inhibition) is calculated from the OD values obtained with the test sample and the Negative Control, which contains no BHV1-specific antibodies.

REAGENTS PROVIDED

| Test Plate, contains 12 microtitre strips with 8 wells each or Test Plate, microtitre plate with 96 wells, coated with inactivated BHV1 antigen | 5 |
| Wash Solution (10x), contains Tween and preservative | 3x 125 ml |
| Sample Diluent, contains Tween and preservative, ready-to-use | 30 ml |
| Positive Control, BHV1-reactive bovine serum in buffer with protein stabilizers and preservative | 3.5 ml |
| Negative Control, BHV1-negative bovine serum in buffer with protein stabilizers and preservative | 3.5 ml |
| Anti-gB-HRP Conjugate, horseradish peroxidase–labelled gB-specific monoclonal antibody in buffer with protein stabilisers and preservatives, ready-to-use | 60 ml |
| TMB (Tetramethylbenzidine) Substrate Solution, ready-to-use, caution! | 60 ml |
| Stop Solution, 0.5 M sulfuric acid, ready-to-use, caution! | 60 ml |

ASSAY PROCEDURE

1. Preparation of the Reagents

1) Wash Solution: Wash Solution (10x), bottle 2, dilute 1:10 with distilled water, e.g., for one Test Plate dilute 50 ml Wash Solution (10x) in 450 ml distilled water and mix.
2) Serum and plasma: Fresh, refrigerated or previously frozen serum or plasma samples may be used. Be sure to change pipette tips for each sample.
3) Milk: Milk samples have to be defatted prior to testing. Centrifuge whole milk samples for 10 min at 3000 x g and 10 °C or store samples cool at 2-8 °C overnight. Then remove the cream. Be sure to change pipette tips for each sample.

2. Assay Steps

A. Test Procedure for serum and plasma
Bring all reagents to room temperature (18-25 °C) before use and mix gently by swirling.
1) Record the positions of the controls and samples in a test protocol.
2) Pipette 50 µl ready-to-use Sample Diluent into the Test Plate wells.
3) Add 50 µl Negative Control and Positive Control, respectively, into appropriate duplicate wells and mix by repeated liquid aspirating and dispensing or by using a plate shaker.
4) Add 50 µl sample into remaining wells and mix by repeated liquid aspirating and dispensing or by using a plate shaker. Carefully cover the Test Plate.
5) Incubate for 2 h at 37 °C or over night at room temperature (18-25 °C) and then empty the wells by aspiration or tapping.
6) Rinse each well 5x with 300 µl of prepared Wash Solution. Remove the buffer after each rinse.
7) Add 100 µl ready-to-use anti-gB-HRP Conjugate to each well.
8) Incubate for 60 min at room temperature (18-25 °C) and then empty the wells by aspiration or tapping.
9) Rinse each well 5x with 300 µl of prepared Wash Solution. Remove the buffer after each rinse.
CALCULATION & EVALUATION

1. Calculation
   a. Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).
   b. The blocking value is calculated according to the following equation:
      \[ \text{% blocking} = \frac{\text{MV OD}_{\text{NC}} - \text{OD}_{\text{Sample}}}{\text{MV OD}_{\text{NC}}} \times 100 \]

2. Evaluation for 2h Sample Incubation
   a. Samples with blocking values < 45 % are negative. Specific antibodies to BHV1 could not be detected.
   b. Samples with blocking values ≥ 45 % and < 55 % are suspect. It is recommended to re-test animals with suspect results.
   c. Samples with blocking values ≥ 55 % are positive. Specific antibodies to BHV1 were detected.

3. Evaluation for Overnight Sample Incubation
   a. Samples with blocking values < 55 % are negative. Specific antibodies to BHV1 could not be detected.
   b. Samples with blocking values ≥ 55 % and < 65 % are suspect. It is recommended to re-test animals with suspect results.
   c. Samples with blocking values ≥ 65 % are positive. Specific antibodies to BHV1 were detected.

TEST VALIDATION

For the assay to be valid the mean value (MV) of the measured OD values for the Negative Control must be ≥ 0.75; the blocking value calculated from the mean value (MV) of the measured OD values for the Positive Control must be ≥ 80 %.

In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

REFERENCES