May 2018

cattletype® BHV1 gE Ab Handbook

For detection of antibodies to glycoprotein E of Bovine Herpesvirus 1

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-B 664

REF 5 plates (cat. no. CT270203)

REF 20 plates (cat. no. CT270205)

INDICAL BIOSCIENCE GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

INDICAL BIOSCIENCE

Contents

Kit contents

cattletype BHV1 gE Ab **Cat. no.** **Number of plates**	**(5)** **CT270203** **5**	**(20)** **CT270205** **20**
Test Plate: microtiter plate with 96 wells, coated with inactivated BHV1 antigen	5	20
Sample Diluent, ready to use	1 x 30 ml	1 x 125 ml
Negative Control, ready to use	1 x 3.5 ml	2 x 3.5 ml
Positive Control, ready to use	1 x 3.5 ml	2 x 3.5 ml
Wash Buffer, 10x concentrate	3 x 125 ml	2 x 500 ml
Conjugate, ready to use	1 x 60 ml	1 x 240 ml
TMB Substrate, ready to use	1 x 60 ml	1 x 240 ml
Stop Solution, ready to use	1 x 60 ml	1 x 240 ml
Handbook	1	1

Intended use

The cattletype BHV1 gE Ab is an enzyme immunoassay (ELISA). It is intended for the detection of antibodies to glycoprotein E of Bovine Herpesvirus 1 (BHV1) in serum, plasma, and milk samples from cattle infected with BHV1. The kit is approved by the Friedrich-Loeffler-Institute and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-B 664) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols

 Legal manufacturer

 Lot number

 Use by date

 Temperature limitations for storage

 Handbook

 Catalog number

 Material number

 For cattle samples

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of cattletype BHV1 gE Ab is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the cattletype BHV1 gE Ab ELISA should be stored at 2-8°C and are stable until the expiration date stated on the label.

Wash Buffer (10x), Sample Diluent and Stop Solution may be stored at room temperature (18-25°C) to avoid salt crystallization. If test strips are provided with the kit, store the remaining test strips in the re-sealed foil pouch with desiccant at 2–8°C until next use. The test strips can be stored for at least 6 weeks after opening the plate pouch.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available from your local sales representative or by Email request under compliance@indical.com.

CAUTION: The Stop Solution contains 0.5 M sulfuric acid.

All sample residues and objects that have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Introduction

The cattletype BHV1 gE Ab is a sensitive assay for the detection of antibodies to glycoprotein E (gE) of Bovine Herpesvirus 1 (BHV1) in serum, plasma, and milk samples. BHV1 is the causative agent of Infectious Bovine Rhinotracheitis (IBR) - a respiratory disease that causes tracheitis, rhinitis, and fever. In addition, BHV1 infection can cause Infectious Pustular Vulvovaginitis (IPV), balanoposthitis and abortions.

Clinical disease is often followed by latent BHV1 infection. Reactivation of the virus can cause the infection to spread in the herd.

Standard serological methods cannot distinguish between naturally infected and vaccinated animals with the exception of IBR vaccines, which do not contain gE viral protein and therefore allow serological differentiation. cattletype BHV1 gE Ab specifically detects antibodies to gE and does not react with antibodies from gE-deleted vaccines. Therefore, this method identifies animals that have been infected with BHV1 field strains or vaccinated with non-gE-deleted IBR vaccines.

Principle

cattletype BHV1 gE Ab is a blocking ELISA. The Test Plate is coated with inactivated BHV1 antigen. During sample incubation, BHV1-specific antibodies bind to the immobilized antigen. Unbound material is removed by rinsing.

The HRP-labeled, gE-specific monoclonal antibody conjugate is added, which cannot bind to the BHV1 antigen while its antigenic determinant is blocked by antibodies in the test sample. Unbound anti-gE-HRP conjugate is rinsed out. A color reaction is initiated 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 gE-specific antibodies.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Beakers
- Measuring cylinders
- Pipets (adjustable)
- Multichannel pipets (adjustable)
- Aluminum or adhesive foil for covering the Test Plate
- Optional: Device for delivery and aspiration of Wash Buffer
- Microtiter plate absorbance reader
- Distilled water

Important notes

General precautions

The user should always pay attention to the following:

- Do not expose the TMB Substrate Solution to intense light or to sunlight when performing the test.
- Components of the test kit should not be contaminated or mixed with components from other batches.
- Do not use the components of the test kit past the expiration date.
- Water from ion-exchange systems used for diluting the Wash Buffer (10x) may interfere with the assay if not pure enough. Use double-distilled water or highly purified water (Milli-Q®).
- For accurate test results, it is essential to use clean glassware and to pipet and rinse carefully and strictly adhere to the incubation times when performing the test.

Protocol: ELISA test procedure

Important points before starting

- Please read „Important notes“ on page 8 before starting.

Things to do before starting

- Bring reagents to room temperature (18-25°C) immediately before use. In case of precipitated salt crystals in the Wash Buffer (10x), dissolve by gentle swirling and warming.
- Dilute Wash Buffer (10x) 1:10 in distilled water. For example, for one Test Plate dilute 50 ml Wash Buffer (10x) in 450 ml distilled water and mix.
- Serum/ plasma samples: Fresh, refrigerated or previously frozen serum or plasma samples may be used.

Preparation of milk samples

Prior to sample analysis, milk samples have to be defatted. Centrifuge whole milk samples for 10 min at 3000 x g at 10°C or store samples at 2-8°C overnight. Then remove the cream.

Test procedure for serum and plasma samples

Please read „Things to do before starting“, page 9.

Procedure

1. Pipet 50 µl of ready to use Sample Diluent into the Test Plate wells.
2. Pipet 50 µl Negative Control (in duplicate) and Positive Control (in duplicate) into appropriate wells and mix.
3. Pipet 50 µl of the samples into remaining wells and mix.

   Note: Record the positions of the controls and samples in a test protocol. Mix by either using a plate shaker or by repeated liquid aspirating and dispensing. Cover the Test Plate.
4. Incubate overnight (16-22 h) at room temperature (18-25°C).
5. Remove solution from the wells by aspiration or tapping.
6. Rinse each well 5x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
7. Pipet 100 µl ready to use Conjugate to each well and incubate for 30 min at room temperature (18-25°C).
8. Remove solution from wells by aspiration or tapping.
9. Rinse each well 5x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
10. Pipet 100 µl TMB Substrate Solution to each well.
11. Incubate for 10 min at room temperature in the dark. Begin timing after the first well is filled.
12. Stop the reaction by adding 100 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.

13. Measure the OD in the plate reader at 450 nm within 20 min after stopping the reaction.

    Measuring at a reference wavelength (620–650 nm) is optional.

Test procedure for milk samples

Please read „Things to do before starting“, page 9.

Procedure

1. Pipet 100 µl Negative Control (in duplicate) and Positive Control (in duplicate) into appropriate wells.
2. Pipet 100 µl of defatted milk samples into remaining wells.

   Note: Record the positions of the controls and samples in a test protocol. Cover the Test Plate.
3. Incubate overnight (16-22 h) at room temperature (18-25°C).
4. Remove solution from the wells by aspiration or tapping.
5. Rinse each well 5x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
6. Pipet 100 µl ready to use Conjugate to each well and incubate for 30 min at room temperature (18-25°C).
7. Remove solution from wells by aspiration or tapping.
8. Rinse each well 5x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
9. Pipet 100 µl TMB Substrate Solution to each well.
10. Incubate for 10 min at room temperature in the dark. Begin timing after the first well is filled.

11. Stop the reaction by adding 100 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.
12. Measure the OD in the plate reader at 450 nm within 20 min after stopping the reaction.

    Measuring at a reference wavelength (620–650 nm) is optional.

Data interpretation

Validation criteria

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Negative Control (NC) must be ≥ 0.5.
- The blocking value calculated from the MV of the measured OD value for the Positive Control (PC) must be ≥ 75%.

In case of invalid assays, the test should be repeated after carefully reading the instructions for use.

Calculation

Calculate the MV of the measured OD for the Negative Control (NC) and the Positive Control (PC).

Calculation for serum and plasma samples

The blocking value is calculated according to the following equation:

$$\% \text{ blocking} = \frac{\text{MV OD}_{NC} - \text{OD}_{sample}}{\text{MV OD}_{NC}} \times 100$$

Calculation for milk samples

The blocking value is calculated according to the following equation:

$$\% \text{ blocking} = \frac{(\text{MV OD}_{NC} * 2) - \text{OD}_{sample}}{(\text{MV OD}_{NC} * 2)} \times 100$$

Interpretation of the results

Data interpretation for serum and plasma samples

- Samples with blocking values < 40% are negative.
  Specific antibodies to BHV1 gE could not be detected.
- Samples with blocking values ≥ 40% and < 50% are suspect.
  It is recommended to retest animals with suspect results.
- Samples with blocking values ≥ 50% are positive.
  Specific antibodies to BHV1 gE were detected.

Data interpretation for milk samples

- Samples with blocking values < 35% are negative.
  Specific antibodies to BHV1 gE could not be detected.
- Samples with blocking values ≥ 35% are positive.
  Specific antibodies to BHV1 gE were detected.

Note: In case of negative test results from individual milk samples, it is recommended to retest serum or plasma samples from those animals, to verify the individual animal BHV-1 status. Testing pool or bulk milk samples, the sensitivity can be increased by using suitable protocols for concentration of immunoglobulin from milk.

INDICAL offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens.
Visit **www.indical.com** for more information about bactotype, cador, cattletype, flocktype, pigtype and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective INDICAL kit handbook or user manual.

Notes

Notes

Notes

Limited License Agreement for cattletype BHV1 gE Ab

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. INDICAL grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.indical.com. Some of these additional protocols have been provided by INDICAL users for INDICAL users. These protocols have not been thoroughly tested or optimized by INDICAL. INDICAL neither guarantees them nor warrants that they do not infringe the rights of third-parties.

2. Other than expressly stated licenses, INDICAL makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.

3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

4. INDICAL specifically disclaims any other licenses, expressed or implied other than those expressly stated.

5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. INDICAL may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.indical.com.

Change index

Handbook	Version	Change
HB-1833-007	May 2018	INDICAL design

Quick guide for cattletype BHV1 gE Ab

Sample dilution:

Serum, plasma 1:2, mix well Milk undiluted

Step	Protocol
1. Sample	100 µl/ well
2. Incubation	Overnight (16-22h) at room temperature (RT)
3. Wash	5 x 300 µl
4. Conjugate	100 µl/ well
5. Incubation	30 min at RT
6. Wash	5 x 300 µl
7. TMB	100 µl/ well
8. Incubation	10 min at RT
9. Stop	100 µl/ well
10. Read	450 nm

Data interpretation

	Negative	Suspect	Positive
Serum, plasma	< 40%	≥ 40% and < 50%	≥ 50%
Milk	< 35%	-	≥ 35%

INDICAL BIOSCIENCE

Ordering: **www.indical.com/contact**
Technical Support: **support@indical.com**
Website: **www.indical.com**