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cattletype[®] BHV1 gB Ab Handbook

For detection of antibodies to glycoprotein B
of Bovine Herpesvirus 1

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-B 491

REF 5 plates (cat. no. CT270043)

REF 20 plates (cat. no. CT270045)



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Kit contents

cattletype BHV1 gB Ab	(5)	(20)
Cat. no.	CT270043	CT270045
Number of plates	5	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
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Intended use

The cattletype BHV1 gB Ab is an enzyme immunoassay (ELISA). It is intended for the detection of antibodies to glycoprotein B of Bovine Herpesvirus 1 (BHV1) in serum, plasma, and milk samples from cattle. The kit is approved by the Friedrich-Loeffler-Institute and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-B 491) 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 gB Ab is tested against predetermined specifications to ensure consistent product quality.

Storage

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

Wash Buffer (10x) 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 gB Ab is a highly sensitive solution for the detection of antibodies to glycoprotein B of Bovine Herpesvirus 1 (BHV1). BHV1 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 cattletype BHV1 gB Ab permits the detection of anti-BHV1 antibodies in serum, plasma, and milk samples of cattle infected with BHV1 or vaccinated with a glycoprotein B containing vaccine.

Principle

cattletype BHV1 gB 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, gB-specific monoclonal antibody is added, which cannot bind to the BHV1 antigen while its antigenic determinant is previously occupied by antibodies in the test sample. Unbound anti-gB-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-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
- Tubes or plates for diluting the samples
- 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.

Note: After sample preparation using a suitable concentration protocol, bulk milk samples can be tested with cattletype BHV1 gB Ab. The assay is validated for testing pools of up to 50 milk samples after bulk milk sample preparation using the cattletype Milk Prep Kit.

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 for 2 hours at 37°C or overnight (12-18 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 60 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 (12-18 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 60 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 μ l 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 ≥ 0.6 .
- The blocking value calculated from the MV of the measured OD value for the Positive Control (PC) must be $\geq 75\%$.

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).

The blocking value is calculated according to the following equation:

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

Interpretation of the results

- Samples with blocking values $< 45\%$ are negative.
Specific antibodies to BHV1 could not be detected.
- Samples with blocking values $\geq 45\%$ and $< 55\%$ are suspect.
It is recommended to retest animals with suspect results.
- Samples with blocking values $\geq 55\%$ are positive.
Specific antibodies to BHV1 were detected.

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, cadon, 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.

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Change index

Handbook	Version	Change
HB-1588-006	May 2018	INDICAL design

Quick guide for cattletype BHV1 gB Ab

Sample dilution:

Serum, plasma 1:2, mix well

Milk undiluted

Step	Short protocol	Overnight protocol*
1. Sample		100 µl/ well
2. Incubation	Serum: 2 h 37°C	Overnight (12-18h) at RT
3. Wash		5 x 300 µl
4. Conjugate		100 µl/ well
5. Incubation		60 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

* If using **milk samples**, incubation **must** be carried out overnight.

Data interpretation

Negative	Suspect	Positive
< 45%	≥ 45% and < 55%	≥ 55%