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cattletype[®] BHV1 gE Ab Handbook

 5 (catalog no. 270203)

 20 (catalog no. 270205)

For detection of antibodies to glycoprotein
E of bovine herpesvirus 1

Registered in accordance with § 17c of the German
Law on Animal Diseases (FLB 664)

 270203, 270205



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Kit Contents

<i>cattletype</i> BHV1 gE Ab	(5)	(20)
Catalog no.	270203	270205
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)	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 registered in accordance with § 17c

of the German Law on Animal Diseases (Flu-B 664) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols

	<N>	Contains reagents for <N> plates
		Legal manufacturer
		Lot number
		Use by date
		Temperature limitations for storage
		Handbook
		Catalog number
		Material number
		For cattle samples

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 online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view and print the SDS for each QIAGEN kit and kit component.



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.

Quality Control

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

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
- 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 10 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 11.

Procedure

1. Pipet 50 μ l of ready to use Sample Diluent into the Test Plate wells.
2. Pipet 50 μ l Negative Control (in duplicate) and Positive Control (in duplicate) into appropriate wells and mix.
3. Pipet 50 μ l 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 μ l of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
7. Pipet 100 μ l 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 μ l of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.

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10. Pipet 100 μ l 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 μ l 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 11.

Procedure

1. Pipet 100 μ l Negative Control (in duplicate) and Positive Control (in duplicate) into appropriate wells.
2. Pipet 100 μ l 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 μ l of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
6. Pipet 100 μ l 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 μ l of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
9. Pipet 100 μ l 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 ≥ 0.5 .
- 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).

Calculation for serum and plasma samples

The blocking value is calculated according to the following equation:

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

Calculation for milk samples

The blocking value is calculated according to the following equation:

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

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 $\geq 40\%$ and $<50\%$ are suspect.
It is recommended to retest animals with suspect results.
- Samples with blocking values $\geq 50\%$ 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 $\geq 35\%$ 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.

Troubleshooting Guide

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit www.qiagen.com).

Appendix: Quick Guide

Sample dilution:

Serum/plasma 1:2, mix well

Milk undiluted

Step	Protocol
1. Sample	100 µl/well
2. Incubation	Overnight (16–22 h) at 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%

Ordering Information

Product	Contents	Cat. no.
<i>cattletype</i> BHV1 gE Ab (5)	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	270203
<i>cattletype</i> BHV1 gE Ab (20)	For 1920 reactions: 20 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	270205
Related products		
<i>cattletype</i> BHV1 gB Ab (5)	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	270043
<i>cattletype</i> BHV1 gB Ab (20)	For 1920 reactions: 20 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	270045
<i>cattletype</i> MAP Ab (5)	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	270803
<i>cattletype</i> MAP Ab (20)	For 1920 reactions: 20 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	270805

Product	Contents	Cat. no.
<i>cattletype</i> Milk Prep Kit (50)	Precipitation Reagent, Neutralization Buffer, Matrix, Elution Buffer, Spin Filters, Collection Tubes	271906

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