CATTLETYPE® BHV1 gB Ab

ELISA Test Kit for the Detection of Antibodies to Bovine Herpesvirus 1

Labor Diagnostik
Leipzig

Instructions for Use

In vitro Diagnostic Kit for cattle
registered in accordance with § 17 c of the German Law on Animal Diseases
Registration No.: FLI-B 491

Applications

The CATTLETYPE® BHV1 gB Ab 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 with CATTLETYPE® Milk Prep (Cat. No. 03-102), pool milk samples can be tested, too.

General Information

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 CATTLETYPE® BHV1 gB Ab ELISA permits the detection of anti-BHV1 antibodies in serum, plasma and milk samples of cattle infected with BHV1 or vaccinated with a glycoprotein B (gB)-containing vaccine.

Description of the Test Principle

The CATTLETYPE® 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.

Ordering Information

CATTLETYPE® BHV1 gB Ab	5x 96 Tests	Cat. No. 03-101/5
	20x 96 Tests	Cat. No. 03-101/20

Reagents

		5 plate kit	20 plate kit
1.	Test Plate, contains 12 microtitre strips with 8 wells each or Test Plate, microtitre plate with 96 wells, coated with inactivated BHV1 antigen	5	20
2.	Wash Solution (10×), contains Tween and preservative	3x 125 ml	1.0 l
3.	Sample Diluent, contains Tween and preservative, ready-to-use	30 ml	125 ml
4.	Positive Control, BHV1-reactive bovine serum in buffer with protein stabilisers and preservative	3.5 ml	2x 3.5 ml
5.	Negative Control, BHV1-negative bovine serum in buffer with protein stabilizers and preservative	3.5 ml	2x 3.5 ml
6.	Anti-gB-HRP Conjugate, horseradish peroxidase–labelled gB-specific monoclonal antibody in buffer with protein stabilisers and preservatives, ready-to-use	60 ml	240 ml
7.	TMB (Tetramethylbenzidine) Substrate Solution, ready-to-use, caution!	60 ml	240 ml
8.	Stop Solution, 0.5 M sulfuric acid, ready-to-use, caution!	60 ml	240 ml

Additional Material and Equipment Required

Beakers, measuring cylinders, analytical pipettes, multichannel pipettes, disposable pipette tips, pipetting troughs, lid, aluminium foil or adhesive for covering the Test Plate, poss. plate shaker, device for delivery and aspiration of wash solution, microtitre plate spectrophotometer, distilled water

Storage, Precautions and Warnings

Store the reagents at 2-8 °C and only bring them to room temperature (18-25 °C) immediately before use. Wash Solution (10x, bottle 2) and Stop Solution (bottle 8) may be stored at room temperature. Diluted Wash Solution should be stored for a maximum of 24 hours at room temperature or 1 week at 2-8 °C. 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 at least for 6 weeks after opening the plate pack.

The test should be performed by persons qualified for laboratory work. Store the TMB Substrate Solution in the dark and do not expose it to intense light or to sunlight during the performance of the test. The 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 expiration date. Water from ion-exchange systems used for diluting the Wash Solution (10x) may interfere with the assay if not pure enough. Water of the quality of double distilled water or highly purified water (Milli-Q) is suitable.

To guarantee the precision of the results, it is absolutely essential to follow the usual precautions for ELISA procedures, including the use of clean glass devices, careful pipetting and rinsing during the test, and strict adherence to the indicated incubation times.
Stop solution contains 2.5 % sulfuric acid and can cause irritation or cauterization, TMB Substrate Solution can cause skin irritation, caution!
All sample residues and objects which have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Preparation of the Reagents

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.

Serum and plasma:

Fresh, refrigerated or previously frozen serum or plasma samples may be used. Be sure to change pipette tips for each sample.

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.

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.

   Recommended template for CATTLETYPE® BHV1 gB Ab ELISA:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	NC	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	PC	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	PC	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

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.
10. Add 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 that the Substrate Solution was added.
13. Measure the optical density (OD) in the spectrophotometer at 450 nm immediately or within 20 min after stopping the reaction. Measuring at a reference wavelength (620-650 nm) is optional.

Test Procedure for milk

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.
   (Recommended template see above.)
2. Pipette 100 µl Negative Control and Positive Control, respectively, into appropriate duplicate wells.
3. Pipette 100 µl of defatted milk samples into remaining wells. Carefully cover the Test Plate.
4. Incubate over night at room temperature and then empty the wells by aspiration or tapping.
5. Rinse each well 5x with 300 µl of prepared Wash Solution. Remove the buffer after each rinse.
6 Add 100 µl ready-to-use anti-gB-HRP Conjugate to each well.
7. Incubate for 60 min at room temperature and then empty the wells by aspiration or tapping.
8. Rinse each well 5x with 300 µl of prepared Wash Solution. Remove the buffer after each rinse.
9. Add 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 that the Substrate Solution was added.
12. Measure the optical density (OD) in the spectrophotometer at 450 nm immediately or within 20 min after stopping the reaction. Measuring at a reference wavelength (620-650 nm) is optional.

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 CATTLETYPE® Milk Prep concentration kit

Test Validation

For the assay to be valid the mean value (MV) of the measured OD values for the Negative Control must be $\geq$ 0.75; the blocking value calculated from the mean value (MV) of the measured OD values for the Positive Control must be $\geq$ 80 %. In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

Calculation

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

2. The blocking value is calculated according to the following equation:

$$\% \text{ blocking} = \frac{\text{MV OD}_{NC} - \text{OD}_{Sample}}{\text{MV OD}_{NC}} \times 00$$

Evaluation for 2h Sample Incubation

- **Samples with blocking values < 45 % are negative.**
  Specific antibodies to BHV1 could not be detected.

- **Samples with blocking values ≥ 45 % and < 55 % are suspect.**
  It is recommended to re-test animals with suspect results.

- **Samples with blocking values ≥ 55 % are positive.**
  Specific antibodies to BHV1 were detected.

Evaluation for Overnight Sample Incubation

- **Samples with blocking values < 55 % are negative.**
  Specific antibodies to BHV1 could not be detected.

- **Samples with blocking values ≥ 55 % and < 65 % are suspect.**
  It is recommended to re-test animals with suspect results.

- **Samples with blocking values ≥ 65 % are positive.**
  Specific antibodies to BHV1 were detected.

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