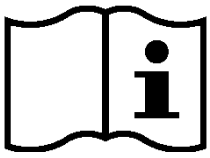

Useŕs Manual

Infectious Bovine Rhinotracheitis Antibody ELISA

An ELISA to detect antibodies to BHV type-1 in bovine serum and plasma samples

REF B1004-AB01

Σ 450

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Gebruik alleen de juiste versie van het protocol dat meegestuurd wordt met de kit.
Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Test kit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

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1. Introduction

Infectious Bovine Rhinotracheitis (IBR) is a severe respiratory herpes virus infection in cattle characterized by tracheitis, rhinitis and fever. IBR is transmitted horizontally by contact with respiratory, ocular and reproductive secretions. IBR also acts as an immunosuppressive, predisposing individuals to secondary bacterial infections. Despite eradication programs for IBR in many parts of the world, infection with IBR remains endemic in many cattle populations resulting in serious economic losses. Serological identification of IBR infected cattle is routinely performed by screening serum samples for antibodies, using serum neutralization tests and indirect ELISA' s. These tests suffer some disadvantages: They are time consuming, insensitive and difficult to read. To detect antibodies in milk samples, more sensitive test systems are required. This ELISA is intended to use as a rapid screening test for the detection of anti-IBR antibodies in serum and plasma samples of infected cattle.

2. Intended use of the test kit

This diagnostic test is intended to identify antibodies against the antigen of BHV-1 virus, in serum and plasma samples.

3. Principle of the test kit

The test is based on the reaction of anti-IBR antibodies with BHV-1 antigen. To this end these BHV-1 antigens are coated to a 96-well microtiter strip-plate. The bovine serum or plasma sample is added to the wells of the coated plate.

Qualitative
The sample is added (diluted 1:2) to the wells of the coated plate.

After incubation, an anti-BHV-1 conjugate is added to compete for the BHV-1 antigen that is coated to the 96-well microtiter strip-plate. The biotinalated conjugate is detected by use of streptividine HRPO. Bound conjugate is made visible by adding substrate/chromogen mix.
Decrease of the color reaction in the wells is directly correlated to the concentration of anti-IBR antibodies in the serum or plasma sample.

4. Contents

- 5 x 12 x 8 Microtiter strips
- 5 x Strip holder
- 1 x 60 ml ELISA buffer
- 1 x 60 ml Biotine conjugated anti-BHV-1 antibodies
- 2 x 35 ml Streptavidine conjugate
- 1 x 0,5 ml Positive control (ready to use) (yellow cap)
- 1 x 1,0 ml Negative control (ready to use) (brown cap)
- 1 x 60 ml Wash solution (200x concentrated) (black cap), **dilute in de-ionized water before use!**
- 1 x 60 ml Substrate A (white cap)
- 1 x 60 ml Substrate B (blue cap)
- 1 x 60 ml Stop solution (yellow cap)
- 5 x Plastic cover seal
- 1 x User's manual

Supplies needed (not included)

- Round-bottomed microtiter plate
- Validated precision pipettes
- Pipette tips and clean containers/tubes (EVL)
- ELISA plate reader

5. Handling and storage of specimens

The kit should be stored at 4°C.
An open packet should be used within 10 days.
Samples may be used fresh or may be kept frozen below -20°C before use.
After first use ready-to-use controls and/or reconstituted controls should be aliquoted immediately and stored at -20°C.
Avoid repeated freezing and thawing as this increases non-specific reactivity.

6. Wash protocol

In ELISA's, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better result.

Manual washing

1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µl wash solution.
3. This washing cycle (step 1 and 2) should be carried out at least 5 times.
4. Turn the plate upside down and empty the wells with a firm vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual wash solution in the wells.
6. Take care that none of the wells dry out before the next reagent is added.

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are aspirated completely and that the wash solution is correctly added, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 5 washing cycles.

7. Preparations

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 min. at room temperature (± 21°C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/ mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4-8°C immediately after use.

8. Test protocol qualitative

Before starting this test read "preparations"

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4°C. and use them within 10 days.

   Wash microtiter strip(s) with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 mega Ohm) water !
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Dilute the **positive control** (yellow cap) **1:2 in ELISA buffer** in a round-bottomed plate (not supplied).
   Example: Add 70µl ELISA buffer (green cap) to well **1A, a**dd 70µl positive control to **well 1A** and mix well.
3. Dilute the **negative control** (brown cap) **1:2 in ELISA buffer** in a round-bottomed plate (not supplied).
   Example: Add 70µl ELISA buffer (green cap) to well **1B**, add 70µl negative control to **well 1B** and mix well.
4. Dilute **each sample 1:2 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied)
   Example: Add 70µl ELISA buffer to **well 1C**, add 70µl sample to **well 1C** and mix well.

5. Take 2 wells as **substrate controls** add only **50µl ELISA buffer** (green cap) to these wells.

6. Transfer 50µl of all dilutions to the coated microtiter strips.
7. Add **50µl biotine conjugated anti-BHV-1 antibodies** to all wells.
8. Seal and incubate for 75 min at 37°C.

9. Wash the strips according to the wash protocol see sub 6.

10. Add **100µl streptavidine conjugate** to all wells.
11. Seal and incubate for 30 minutes at room temperature (± 21°C).

12. Wash the strips according to the wash protocol see sub 6.

13. Mix equal parts of substrate A (white cap) and substrate B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.**

14. Add **100µl substrate solution** to each well.
15. Incubate 10-20 min. in the dark (e.g. cover the wells with a sheet of paper). at room temperature (21°C.). Make sure the negative does not become too dark.

16. Add **50µl stop solution** to each well; mix well.
17. Read the absorbency values immediately (within 10 min!) at 450 nm by using an ELISA reader. **Use the substrate controls as blank.**

NB: if you pipet directly into the coated ELISA plate with only a small number of samples (<6), make sure the first dilution is done in round bottom microtiter plate second step can be done directly in the coated Elisa plate.

9. Precautions

- Handle all biological material as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal, results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

10. Validation of the test

Qualitative:

- The results are valid if the following criteria are met:
  - The mean value (MV) of the measured OD value for the Negative Control (PC) diluted 1:2, must be ≥0.900.
  - The MV of the measured OD value for the Positive Control (NC), diluted 1:, must be ≤0.350.

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

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

  The ratio (S/P) of sample OD to mean OD of the positive control is calculated according to the following equation:

$$S/N = \frac{OD_{sample}}{MVOD_{NC}}$$

The negative control, undiluted, should be lower than 0.250 OD units (450nm) and give an endpoint titer of ≤3.

11. Interpretation of the test results

Qualitative: Positive – Negative

- A sample with the S/N ratio > 0.7 is negative.
  - Specific antibodies to IBR could not be detected.
- A sample with the S/N ratio ≤0.6 is positive.
  - Specific antibodies to IBR were detected.
- A sample with the S/N ratio between the positive and negative value are doubtful.

12. Symbols used with EVL ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructionsforuse	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	Forresearchuseonly	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
Σ	Containssufficient for <n> tests/	Ausreichendfür "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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