Product information

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User's Manual

BLV (Bovine Leukemia Virus) p24/gp51 Ab (serum) ELISA

Enzyme Immunoassay for the quantitative determination of Bovine Leukemia Virus (BLV) p24/gp51 antibodies in serum or plasma.

VET

REF DE2495

Σ 96 wells

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Version 07-01/15
/ DMC
Updated 150113

Please use only the valid version of the package insert provided with the kit.

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1 INTRODUCTION

Serological identification of cattle infected with Bovine Leukaemia Virus (BLV) can be performed by screening for antibodies against the major core protein of BLV, a 24,000 Dalton polypeptide (p24) and against the major envelope antigen, a 51.000 Dalton glycoprotein (gp51).

The agar gel precipitation test (AGPT), and conventional enzyme-linked immunosorbent assays (ELISAs) have proved to be adequate for antibody detection in serum.

However, more sensitive and specific ELISAs' are required for detection of the low antibody titres usually found in milk samples.

An indirect ELISA system has been developed for screening programs. This test enables BLV antibodies to be detected in individual or pooled serum/plasma samples.

The specifications of this test kit are in accordance with the guidelines provided by the European Community (88/406/EC dd. 14-06-1988). According to the same guidelines 10 to 20 samples can be pooled.

2 INTENDED USE OF THE TEST KIT

This diagnostic test system for BLV infected cattle is intended to identify BLV p24 and gp51 antibodies in individual or pooled serum/plasma samples.

In contrast to test systems which make use of polyclonal antibodies or only one gp51 monoclonal antibody, this two monoclonal antibodies mediated ELISA gives a minimum of non-specific reactions.

3 STANDARDISATION

Standardisation against the E4 serum has been developed in such a way that a positive signal is obtained after a dilution of E4 serum (either in buffer or in negative serum) of at least 1:2500.
(Hoff Jorgensen, R., et al., 1989)

4 PRINCIPLE OF THE TEST KIT

The test is based on the reaction of anti-BLV-p24 and anti-BLV-gp51 antibodies in test samples with BLV antigen which have been coated to a 96 well microtiter plate.

After washing, test samples are added to the wells, and if present, anti-BLV-p24 and anti-BLV-gp51 antibodies from the samples will bind to the antigen.

The complex is detected by a horseradish peroxidase (HRPO) conjugated monoclonal antibody directed against bovine IgG.

Colour reaction in the wells is directly related to the presence of BLV-p24 and BLV-gp51 antibodies in the sample.

5 CONTENTS

- 12 x 8 **microtiter strips**
- 1 x **strip holder**
- 1 x 18 mL **ELISA buffer**
- 1 x 12 mL BLV **antigen** (ready to use)
- 1 x 13 mL HRPO-**conjugated anti-species antibody** (ready to use)
- 1 x 0.5 mL **Positive control** (lyophilized)
- 1 x 0.5 mL **Negative control** (lyophilized)
- 1 x 20 mL **Wash solution** (200x concentrated), **dilute in de-ionized water before use!**
- 1 x 8 mL **Substrate A**
- 1 x 8 mL **Substrate B**
- 1 x 8 mL **Stop solution**
- 1 x Plastic cover seal

Supplies needed (not included): Round bottomed microtiter plate

6 HANDLING AND STORAGE OF SPECIMENS

The kit should be stored at 2 °C - 8 °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.

Positive and negative controls may be stored after reconstitution in aliquots at -20 °C and used until the expiry date.

Avoid repeated freezing and thawing as this increases non-specific reactivity.

7 WASH PROTOCOL

In ELISAs, 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 results

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 washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 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 washing solution in the wells.
6. Take care that none of the wells dry out before the next reagent is dispensed

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are aspirated completely and that the washing solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 4 washing cycles.

8 TEST PROTOCOL

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 2 °C - 8 °C and use them within 10 days.
   Wash the microtiter strip(s) with washing solution, according to washing protocol.
   The washing solution provided must be diluted 200 x in de-ionized water!
2. Add 100 µL BLV antigen to all wells to be used. (Store leftover of BLV antigen at -20 °C.)
3. Incubate 60 minutes at 37 °C.
4. Wash 5 times as in step 1.
5. Reconstitute controls with 0.5 mL aqua bidest
   make a dilution 1:30 of the positive and negative **control** in a round bottomed microtiter plate.
6. Add 100 µL negative control (diluted 1:30) and 100 µL positive control (diluted 1:30) to each well.
   Use 3 wells for negative control and 3 wells for positive control.
   (Store leftover of reconstituted controls at -20 °C in aliquots).
7. Make a **dilution 1:30 of serum/plasma samples** (preferable in duplicate) in ELISA buffer in a round bottomed microtiter plate (not supplied).
   Individual or pooled samples (up to 15 samples can be pooled) should be diluted 1:30 in ELISA buffer.
8. Transfer 100 µL of these dilutions to the 90 remaining wells of the coated microtiter plate.
9. Seal and incubate for 60 minutes at 37 °C.
10. Wash as pointed out in wash protocol.
11. Dispense 100 µL of the ready to use HRPO conjugate to all wells.
12. Seal and incubate 60 minutes at 37 °C.
13. Wash as pointed out in wash protocol.
14. Mix equal parts of buffer A and buffer B with gentle shaking. **Prepare immediately before use!**
    Dispense 100 µL substrate solution to each well.
15. Incubate 10-15 minutes at room temperature (21 °C).
16. Add 50 µL stop solution to each well; mix well.
17. Read the absorbency values immediately (within 10 minutes!) at 450 nm.

9 PRECAUTIONS

- Handle all biological material as possible infectious.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer A/B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the 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

In order to confirm appropriate test conditions, the mean absorbency value of the negative control should be lower than 0.250 OD units and the mean OD value of the positive control provided should be higher than 1.0 OD units.

11 INTERPRETATION OF TEST RESULTS

A sample is scored BLV negative if the OD value is below or equal to the average OD value of the negative control plus 0.250 OD units.

Negative: OD samples ≤ OD negative control plus 0.250 OD units.

NOTE:

If a sample does not meet these criteria for being negative, the following protocol is advised:

Serum samples: Confirmation of positive results can be achieved by testing individual serum samples in the Bovine Leukaemia Serum complex-trapping-blocking ELISA test or PCR.

The entire risk as to the performance of these products is assumed by the purchaser. Demeditec shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products.

In case of problems or questions contact Demeditec.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konformitäts-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	For research use only	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
VET	For veterinary use only				
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "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