# Product information

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User's Manual

# BLV (Bovine Leukemia Virus) p24 (Blocking) ELISA

*A monoclonal antibody-mediated complex-trapping-blocking ELISA for the specific detection of Bovine Leukemia Virus p24 antibodies in serum samples.*

REF DE3159

Σ 96 wells

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***Please use only the valid version of the package insert provided with the kit.***
***Verwenden Sie nur die jeweils gültige, im Testkit 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

Serological identification of Bovine Leukemia Virus (BLV)-infected cattle can be performed by screening for antibodies against a 24,000 Dalton polypeptide (p24) of the viral core. The agar gel precipitation test (AGPT), and conventional enzyme-linked immunosorbent assays (ELISA) have proven adequately sensitive and specific for antibody detection in serum and milk. However, more specific ELISA's are required to confirm results obtained by AGPT and convential ELISA's.

## 2 INTENDED USE OF THE TESTKIT

This diagnostic test is intended to identify BLV-p24 antibodies in individual serum samples.

In contrast to tests which make use of polyclonal antibodies, this monoclonal antibody-mediated ELISA gives minimum non-specific reactions.

This ELISA has a similar specificity to the agar gel precipitation test (AGPT), but is more sensitive.

## 3 PRINCIPLE OF THE TEST KIT

Test samples and inactivated BLV antigen are simultaneously added to the wells and incubated.

Without washing, a horseradish peroxidase conjugate, prepared with another monoclonal antibody directed against BLV-p24 is added to the wells.

Blocking of the color reaction in the wells is directly related to the presence of BLV p24 antibodies in the samples.

## 4 CONTENTS

- 12 x 8 **microtiter strips**
- 1 x strip holder
- 1 x 12 mL inactivated BLV **antigen** (Ready To Use)
- 1 x 12 mL Biotin-conjugated anti-BLV monoclonal **antibodies** (Ready to use)
- 1 x 1 mL **Positive control** (Freeze dried)
- 1 x 1 mL **Negative control** (Freeze dried)
- 1 x 0,2 mL 100x **concentrated Conjugate**
- 1 x 12 mL **Conjugate buffer**
- 1 x 60 mL **Wash solution** (200x concentrated), **dilute in de-ionized water before use!**
- 1 x 11 mL **Substrate A**
- 1 x 11 mL **Substrate B**
- 1 x 11 mL **Stop solution**
- 1 x Plastic cover seal

## 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.

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.

## 6 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.

## 7 TEST PROTOCOL

1. Reconstitute directly before use the positive and negative controls in 1 mL PBS (not provided).
2. Open the packet of strips and take out the strips to be used. Leave the remaining strips covered in the plastic and store at 4 °C – 8 °C. Allow all materials used to come to room temperature.

   Wash the microtiter strip(s) with washing solution according to the washing protocol.
   **The provided washing solution must be diluted 200x in de-ionized water!**
3. To three wells of the coated strip add 50 µL of positive control and to another three wells 50 µL of negative control. In addition, add 50 µL of each **undiluted sample** to an individual marked well of the strip.
4. Immediately after addition of the serum samples add 50 µL of inactivated BLV antigen to all wells.
5. Seal and incubate for 30 minutes at 37 °C.
6. Dispense 100 µL of Biotin-conjugated anti-BLV monoclonal antibodies to all wells (**without emptying or washing the plate!**).
7. Seal and incubate for 60 minutes at 37 °C.
8. Wash as pointed out in wash protocol.
9. **Dilute the 100x concentrated Conjugate 1:100 in Conjugate-buffer.**
10. Dispense 100 µL of **diluted Conjugate** to all wells.
11. Seal and incubate for 30 minutes at 37 °C.
12. Wash as pointed out in wash protocol.
13. Mix equal parts of substrate A and substrate B with gentle shaking. **Prepare immediately before use!**
14. Dispense 100 µL of substrate solution to each well.
15. Incubate for 10 - 15 minutes at room temperature (21 °C).
16. Add 50 µL of stop solution to each well; mix well.
17. Read the absorbency values immediately (within 10 minutes!) at 450 nm. Use 620 nm as reference wavelength.

## 8 PRECAUTIONS

- Handle all biological material as though capable of transmittingBLV.
- 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.

## 9 VALIDATION OF THE TEST

In order to confirm appropriate test conditions the mean absorption value of the negative control should be between 1.100 - 1.800 OD units (450 nm).

The positive control should be below half of the negative signal.

## 10 INTERPRETATION OF TEST RESULTS

- A sample is scored **NEGATIVE** if the measured OD value is above 80% of the negative OD.

  Example: OD negative 1.400, 80% = 1.120
  All samples with an OD value above 1.120 are considered negative.

- A sample is scored **POSITIVE** if the measured OD value is below 60% of the negative OD.

  Example: OD negative 1.400, 60% = 0.840
  All samples with an OD below 0.840 are considered positive.

- All samples scored between 80% and 60% of the negative signal are considered **DOUBTFUL**.

  Example: OD negative 1.400, 80% = 1.120, 60% = 0.840
  All OD values between 0.840-1.120 are considered doubtful and should be tested again.

*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-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 | 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 |
| 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 |