

User's Manual


Bovine Leukemia

P24/gp51

Antibody ELISA

A monoclonal mediated antibody sandwich
ELISA to detect antibodies against Bovine
Leukemia Virus p24/gp51 in milk samples

REF B1001-AB02

 480

December 2020

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 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.

Inhoud / Table of Contents / Inhaltsverzeichnis / Tabella die Contenuti / Tabla de Contenidos

1. Introduction.....	3
2. Intended use of the test kit	3
3. Principle of the test kit	3
4. Contents	4
5. Handling and storage of specimens	4
6. Wash protocol	5
7. Preparations	5
8. Test protocol Qualitative.....	6
Before starting this test read “ preparations ”	6
9. Precautions.....	7
10. Validation of the test.....	8
11. Interpretation of the test results.....	8
12. Symbols used with EVL ASSAYS	9

1. Introduction

Identification of cattle infected with Bovine Leukemia 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 immune sorbent assays (ELISA) have proved to be adequate for antibody detection in serum.

However, more sensitive and specific ELISA'S are required for detection of the low antibody titers 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 milk samples (≤ 10 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).

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 milk samples (≤ 10 samples). The sensitivity of the test is adjusted to the standards of the European Community with reference to the E4 serum and meets the requirements for testing milk samples in the European enzootic bovine leucosis (EBL) screening program. 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. 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.

➤ **Qualitative**

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

After washing, milk 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.

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

4. Contents

- 5 x 12 x 8 Microtiter strips coated with monoclonal anti-BLV-p24 and anti-BLV-gp51 antibodies
- 5 x Strip holder
- 2 x 60 ml ELISA buffer
- 1 x 60 ml Inactivated Antigen (lilac cap)
- 1 x 60 ml HRPO conjugated anti-species antibodies
- 1 x 0,5 ml Positive control (freeze dried) (purple cap)
- 1 x 1,0ml Negative control (freeze dried) (silver 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

- Milk samples have to be defatted. Centrifuge whole milk samples for 10 minutes at 3000 x g or store samples at 2°C – 8°C overnight. Then remove the cream.
- 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. Centrifuge whole milk samples for 10 minutes at 3000 x g or store samples at 2°C – 8°C overnight. Then remove the cream.
2. 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.

3. Add **100µl of inactivated BLV antigen** to all wells to be used.
4. Incubate 60 min. at 37°C.
5. Reconstitute directly before use the **positive control** (purple cap) **in 0,5 ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
6. Reconstitute directly before use the **negative control** (silver cap) **in 1,0ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
7. Dilute the **positive control** (purple cap) **starting 1:25 → 1:75 → 1:225 → 1:675 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
Example: - A **pre-dilution** is needed:
 - Add 80µl ELISA buffer to **row 1A**, add 20µl of the positive control to the **well 1A** and mix well.
 - Add 160µl ELISA buffer to **row 2A**,
 - And 120µl to **2B, 2C, 2D**
 - Add 40µl of pre-dilution **well 1A** in the well **2A** and mix well
 - Mix well and transfer 60µl to the well **2B**
 - Mix well and transfer 60µl to the well **2C**
 - Mix well and transfer 60µl to the well **2D**
 - Mix well and discard 60µl.
8. Dilute the **negative control** (silver cap) **1:25 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
Example: - Add 120µl ELISA buffer to **well 2E**, add 5µl of the negative control to the **well 2E** and mix well.
9. Dilute the **each sample 1:1 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
Example: - Add 70µl ELISA buffer to **well 2F**, add 70µl of the de-fatted milk sample to the **well 2F** and mix well.
10. Take 2 wells as **substrate controls** add only **120µl ELISA buffer** (green cap) to these wells.
11. Wash the strips with antigen incubated plate (step 3) according to the wash protocol ^{see sub 6}.

12. Transfer 100µl of all dilutions of **row 2** to the coated microtiter strips, including the substrate controls.
13. Seal and incubate for 60 min at 37°C.
14. Wash the strips according to the wash protocol ^{see sub 6}
15. Add **100µl HRPO conjugated anti-species antibodies** to all wells.
16. Seal and incubate for 60 minutes at 37°C.
17. Wash the strips according to the wash protocol ^{see sub 6}.
18. 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.**
19. Add **100µl substrate solution** to each well.
20. 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 control does not become too dark.
21. Add **50µl stop solution** to each well; mix well.
22. 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 Positive Control (PC) diluted 1:25 must be ≥ 0.750 .
 - The MV of the measured OD value for the Negative Control (NC) diluted 1:25 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:

$$S/P = \frac{OD_{sample} - MV OD_{NC}}{MV OD_{PC} - MV OD_{NC}}$$

11. Interpretation of the test results

Qualitative: Positive – Negative

- A sample with the S/P ratio > 0.28 is positive.
 - Specific antibodies to bovine Leukemia could not be detected.
- A sample with the S/P ratio ≤ 0.28 is negative.
 - Specific antibodies to bovine Leukemia were detected.

12. Symbols used with EVL 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
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	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	Mindesthaltbarkeitsdatum	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

The entire risk as to the performance of these products is assumed by the purchaser. EVL 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 EVL.