

DRG[®] Bovine Leukemia Virus p24-gp51 Ab (serum, milk) (EIA-2496)**Revised 20 Apr. 2010 rm (Vers. 2.0)****For Veterinary Use Only***Please use only the valid version of the package insert provided with the kit.***INTRODUCTION****For Veterinary use only!**

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 (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.

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.

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.

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 pooled milk samples in the European enzootic bovine leukosis (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.

STANDARDIZATION

Standardization 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 milk) of at least 1:2500 (Hoff Jorgensen, R., et al., 1989)

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. To this end, monoclonal anti-BLV-p24 and anti-BLV-gp51 antibodies have been coated to a 96 well microtiter plate.

Inactivated BLV p24/gp51 antigens added to the wells are captured by the coated monoclonal antibodies.

After washing, test 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.

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CONTENTS

- 12 x 8 **microtiter strips** coated with monoclonal anti-BLV-p24 and anti-BLV-gp51 antibodies and saturated with BLV p24 and gp45 antigen.
- 1 x 13 ml **HRPO-conjugate buffer**
- 1 x 0.150 ml concentrated **HRPO**
- 1 x 2 ml inactivated **positive control** serum (ready to use).
- 1 x 2 ml inactivated **negative control** serum (ready to use).
- 1 x 20 ml **wash solution** 200 x concentrated, which must be diluted in deionized water before use!
- 1 x 23 ml **ELISA buffer**
- 1 x 8 ml **substrate buffer A**
- 1 x 8 ml **substrate buffer B**
- 1 x 8 ml **stop solution**
- 1 x Plastic cover seal

HANDLING AND STORAGE OF SPECIMENS

The kit should be stored at +4°C.

After reconstitution the lyophilised reagents should be used immediately.

Positive and negative controls may be stored after reconstitution in aliquots at -25°C and used until the expiry date.

Repeated freezing and thawing must be avoided as this increases non-specific reactivity and decreases specific reactivity.

Fresh samples can be used. However addition of 0.1% sodium azide to the samples has no influence on the results of the test.

PREPARATION OF SPECIMENS

Milk samples - undiluted

For optimal sensitivity pooled milk samples can be tested undiluted.

To avoid false positive reactions defatted samples must be used. Centrifuge the milk samples for 15 min. at 2500 g and take a sample below the fat layer.

Milk samples - diluted

For optimal specificity individual milk samples should be tested undiluted or at a 1:2 dilution.

Pooled milk samples, collected from up to 15 individual animals, can be tested undiluted.

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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 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 by 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 dries 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.

TEST PROTOCOL

1. Wash the microtiter plate with wash solution, according to wash protocol.
The wash solution provided must be diluted 10 x in deionized water!
2. Allow samples to come to room temperature.
Samples and controls should preferably not be diluted in the coated microtiter plate, but in a separate round bottomed microtiter plate.

Add 100 µL negative control and 100 µL positive control to 3 wells each.

Add 100 µL of the preferable undiluted milk samples to the 90 remaining wells of the coated plate.

3. Seal the microtiter plate and incubate for 60 minutes at 37°C.
4. Wash as in 1.
5. *Dilute the concentrated conjugate 1:100 in HRP conjugate buffer.*

Add 100 µL diluted conjugate to all wells.

6. Seal and incubate 60 minutes at 37°C.
7. Wash as in 1.

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8. *Prepare substrate solution immediately before use! Mix equal parts of buffer A and buffer B with gentle shaking.*

Add 100 µL substrate solution to each well.

Incubate for 15 min. (10-20 min. is ok) at room temperature (21°C.).

9. Add 50 µL stop solution to each well.

10. Read the absorbency values immediately, within 10 min. at 450 nm. Use 620 nm as reference wave length.

PRECAUTIONS

- Handle all biological materials as though capable of transmitting BLV.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.
- Do not use components past their 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 microtitre plate and protect it from damage and dirt.

VALIDATION OF THE TEST

In order to confirm appropriate test conditions, the mean absorbency value of the negative control should be lower than 0.150 OD units and the mean OD value of the positive control provided should be higher than 0.800 units.

INTERPRETATION OF TESTRESULTS

A sample is scored BLV negative if the OD value is below or equal to the average OD value of the negative control plus 0.150 OD units.

Negative: OD samples \leq Mean OD negative control plus 0.150 OD units.

Note: If an individual sample does not meet these criteria for being negative, the sample should be retested undiluted, 1:2 and 1:4 diluted.

REFERENCES / Literature

Hoff Jorgensen, R., 1989, An international comparison of different laboratory tests for the diagnosis of bovine leukosis: suggestions for international standardization.

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



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Symbols used with DRG 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
	For veterinary use only				
	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	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