DRG® Bovine Leukemia Virus (Blocking) p24 Ab (EIA-3159)

REVISED 18 JUNE 2008 (VERS. 1.0) **FOR VETERINARY USE ONLY**

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 conventional ELISA's.

INTENDED USE

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.

PRINCIPLE

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.

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**
- 1 x 1 mL **Positive control** (Freeze dried)
- 1 x 1 mL **Negative control** (Freeze dried)
- 1 x 12 mL Streptavidin-HRPO-**conjugate**
- 1 x 20 mL **Wash solution** (200X concentrated), **dilute in de-ionized water before use!**
- 1 x 7 mL **Substrate A**
- 1 x 7 mL **Substrate B**
- 1 x 7 mL **Stop-solution**
- 1 x Plastic cover seal

Each kit contains sufficient reagents to perform 96 tests.

HANDLING AND STORAGE OF SPECIMENS

The ELISA should be stored at 4-8˚C. An unopened package can be used until the expiry date. An opened package can be used if the requirements, mentioned in the validation (see 9), are fulfilled. If not fulfilled the test can no longer be used.

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

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.

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 results.

1.1 Manual washing

1. The wash-solution is 200X concentrated, **dilute in de-ionized water before use!**
2. Empty each well by turning the microtiter plate upside-down followed by a firm vertical downward movement to remove the contents of the wells.
3. Fill all the wells with 250 µL of diluted washing solution.
4. This washing cycle (2 and 3) should be carried out ***at least 4 times!***
5. Turn the plate upside down and empty the wells with a firm vertical downward movement.
6. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual washing solution in the wells.
7. Take care that none of the wells dries out before the next reagent is dispensed.

1.2 Washing with automatic equipment

The wash-solution is 200X concentrated, **dilute in de-ionized water before use!**

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!***

PRECAUTIONS

- Handle all biological materials as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke, prepare foods or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, through contact with skin or when swallowed; observe when handling the substrate.
- Do not use components past the expiry date and do not mix components from different serial lots together.
- 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.

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 4-8˚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 one well of the coated strip add 50 µL of positive control and to another well 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 in 2.
9. Dispense 100 µL of Streptavidin-HRPO-conjugate to all wells.
10. Seal and incubate for 30 minutes at 37˚C.
11. Wash as in 2.
12. Mix equal parts of substrate A and substrate B with gentle shaking. **Prepare immediately before use!**
13. Dispense 100 µL of substrate solution to each well.
14. Incubate for 10 - 15 minutes at room temperature (21°C).
15. Add 50 µL of stop solution to each well; mix well.
16. Read the absorbency values immediately (within 10 minutes!) at 450 nm. Use 620 nm as reference wavelength.

VALIDATION OF THE TEST

In order to confirm appropriate test conditions
the mean absorption value of the negative control should be between 1.400 - 2.500 OD units (450 nm).
The positive control should be below half of the negative signal.

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 % = 0.560
All samples with an OD below 0.560 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% = 0.560
All OD values between 0.560 - 1.120 are considered doubtful and should be tested again.

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

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SYMBOLS USED WITH DRG ASSAY´S

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

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..