# DRG® Avian Leucosis Virus-p27 Antigen ELISA (EIA-2499)

**Revised 8 Mar. 2011 rm (Vers. 4.1)** **For Veterinary Use Only**

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

***This kit is intended for Veterinary Use Only. Not for use in diagnostic procedures on humans.***

## 1 INTRODUCTION

Avian Leucosis Virus (ALV) can be detected by screening for ALV-p27 antigen, a 27,000 dalton polypeptide which is the major core protein of the virus.

In the past indirect or direct complement fixation tests were mainly applied, but recently more sensitive enzyme linked immunosorbent assays (ELISAs) have been introduced. Preparation of the required antisera, however, has always been erratic so that batch-to-batch variations and cross-reactions with chicken proteins sometimes occurred.

In contrast to other test systems which make use of polyclonal antibodies, this ELISA test kit incorporates monoclonal antibodies which give a minimum of non-specific reactions.

The availability of this kit may facilitate larger scale testing of ALV shedding in avian leucosis eradication programs. In addition the increased sensitivity (20 to 40 times) and specificity of the kit will enable improvement of current laboratory techniques in avian retrovirus research.

## 2 INTENDED USE OF THE TESTKIT

This diagnostic test system is intended to identify ALV-p27 antigen in serum or egg samples, and in individual or pooled samples.

## 3 STANDARDISATION

To standardise the ALV-p27 ELISA, an ALV positive and negative control have to be tested.

The positive control should have an OD > 0.800 and the negative control should have an OD < 0.300.

## 4 CONTENTS

- 1 x 96-well **microtiter plates** coated with monoclonal anti-p27 antibody
- 1 x 13 mL **HRPO-conjugate buffer**
- 1 x 0,3 mL concentrated HRPO **conjugate**, dilute 1:100
- 1 x 1 mL inactivated ALV **positive control** (freeze-dried)
- 1 x 1 mL inactivated ALV **negative control** (freeze-dried)
- 1 x 20 mL **wash solution 200 x** concentrated (must be diluted in deionized water before use!)
- 1 x 22 mL **ELISA buffer**
- 1 x 7 mL **substrate buffer A**
- 1 x 7 mL **substrate buffer B**
- 1 x 8 mL **stop solution**

## 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 have to 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 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. Dilute 100 µL of the samples (egg or serum) to be tested with 100 µL ELISA buffer in round bottomed microtiter plate.
2. Transfer 100 µL of the sample dilution to each well of the coated microtiter plate.

   Reconstitute the positive and negative control with 1 mL PBS **(not included).**

   Add 100 µL of positive control (in duplicate) to separate wells (well A1 and B1) and
   100 µL of negative control (in duplicate) to separate wells (C1 and D1).
3. Seal and incubate for 90min at 37 °C.
4. Wash as pointed out in wash protocol.

5. Diluted the concentrated HRPO conjugate 1:100 in conjugate buffer.
   Add 100 µL conjugate to all wells.
   Seal and incubate for 1 hour at 37 °C.
6. Wash pointed out in wash protocol.
7. Mix equal parts of buffer A and B together with gentle shaking!
   (Prepare immediately before use!)
   Dispense 100 µL substrate solution to each well.
   Incubate for 10-15 min. at room temperature (21 °C.)
8. Stop the reaction by adding 50 µL stop solution to each well and mix well.
9. Read the absorbency values immediately (within 10 min!) at 450nm.
   Use as a reference a wave length of 620nm.

## 8 PRECAUTIONS

- Handle all biological material as though capable of transmitting ALV.
- 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 OD of the positive control should be > 0.800 OD (450 nm).

Negative control should be < 0,250 OD (450 nm).

## 10 INTERPRETATION OF TEST RESULTS

A sample is considered **positive** when the measured extinction is higher than 2 times the OD of the negative control.

A sample is considered **doubtful** when the measured extinction is between the OD of the negative control and 2 times the value of the negative control

*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.*