

# Avian Leukosis Virus (P27) Antigen ELISA Kit

## ALV P27 Ag Test

**Product Number:** E21161

**Product Unit:** 1 plate, 96T / 2 plates, 192T / 5plates, 480T

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#### Manufacturer Information

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## 1. Introduction

Avian sarcoma leukosis virus (ASLV) is an endogenous retrovirus that infects and can lead to cancer in chickens; experimentally it can infect other species of birds and mammals. ASLV replicates in chicken embryo fibroblasts, the cells that contribute to the formation of connective tissues. Lymphoid leukosis is the most common form of this disease and with typical presentation of gradual onset, persistent low mortality, and neoplasia of the bursa. Subgroups A, B, E and J are the major subgroups of avian leukosis virus (ALV) infecting chickens. ALV infection has become endemic in many countries and has a significant negative effect on the poultry industry.

As the capsid protein, P27 is the group-specific antigen of ALV and has many viral antigen sites, which enable the easy detection of the virus itself, and based on which ELISA or lateral flow immunoassay were further developed for laboratory testing purpose.

## 2. Description of Test

The current ALV-Ag P27 ELISA kit is designed to detect P27 protein in various chicken samples. The 96well microtiter plate was precoated with a P27 protein specific monoclonal antibody. During testing, samples are added into the microplate wells, in which the precoated antibody will capture the ALV in sample and formed antigen-antibody complex. None specific antigens are discarded by a washing step. Then another anti-P27 monoclonal antibody conjugate labeled with horseradish peroxidase (HRP) is added into each well, and further forms antibody-antigen-antibody complexes. After another washing step to remove unreacted conjugate, substrate is added and a blue color will be developed if P27 antigen is present. The enzyme reaction is stopped and the OD450nm value is measured. The measured intensity is positively proportional to the amount of P27 antigen present in the sample.

This ELISA kit can be used to detect ALV in **egg albumen, meconium** and **cloacal swabs**.

## 3. Precautions

- Store the kit at 2-8°C, Check the lot number and expiration date before use.
- Bring the test kit to room temperature before use. For example, take it out from the cold storage and put at room temperature for at least 30min.
- The stop solution in the kit is acidic, please make sure do not touch it with your hand or skin.
- The component of the kit is noninfectious, but the field sample shall be treated as potentially infectious. Please handle all these materials properly according to your lab regulations.
- After experiment, all lab materials shall be handled properly according to local regulations.

## 4. Limitations of Test

This ELISA kit is currently designed for research purpose. We recommend validating in your own lab with different methodologies to confirm the performance. If it is not used for the mentioned purpose, please contact us for help.

## 5. Reagent Provided

The kit contains the following items.

| Item No. | Description                                             | Quantity  |
|----------|---------------------------------------------------------|-----------|
| 1        | Microplate pre-coated with anti-P27 monoclonal antibody | 5 plates  |
| 2        | Positive Control                                        | 1 X 2 ml  |
| 3        | Negative Control                                        | 1 X 2 ml  |
| 4        | Anti-P27 monoclonal antibody HRP conjugate              | 1 X 60 ml |
| 5        | TMB substrate                                           | 1 X 60 ml |
| 6        | Stop solution                                           | 1 X 60 ml |
| 7        | 25X Wash buffer                                         | 1 X 60 ml |
| 8        | Kit instruction                                         | 1set      |

## 6. Instrument Required

- ELISA reader
- Micropipette 20-200ul
- Micropipette Multi-Channel 50-300ul

## 7. Reagent Preparation

- **Wash buffer:** dilute the 25xWash buffer provided in the kit with deionized water in the volume ratio of 1:24. For example, 1ml 25xWash buffer + 24ml deionized water  
The diluted wash buffer can be stored at room temperature for 2 weeks.

**No other reagent is required. Please remember to return all kit component to room temperature before use.**

## 8. Sample Preparation

- **Egg albumen:** use for assay directly without any pre-treatment.
- **Cloacal swab / Meconium:** dissolve or extract with sample buffer. Repeated freezing and thawing is recommended to improve the ALV antigen extraction.

## 9. Assay Procedure

- 1) Make sure the kit and all test samples are returned to room temperature before use. Shake each reagent gently before adding into the well.
- 2) Open the kit, read the kit instruction carefully to make sure all technical points are understood clearly.
- 3) Take the microplate from the zip-bag, and take needed microwells, store the rest into the zip-bag. Make marks of the plate layout. Running the test in duplicated wells is recommended to minimize operational error.
- 4) Add Positive control: add 100ul positive control into the wells.
- 5) Add Negative control: add 100ul negative control into the wells.
- 6) Add Sample: add 100ul sample into the wells.
- 7) Incubation: cover the plate with plate cover and incubate at room temperature (22-27 °C) for 60min.
- 8) Washing: pour the liquid out from the wells and wash with wash buffer (300ul per well) for 3 times. Tap the residue liquid against absorbent paper to make sure the plate is dry after washing.

9) Add enzyme conjugate: add 100ul of HRP conjugate into each well. Cover the plate again and then incubate at room temperature again for 60min.

10) Washing: repeat the washing step again.

11) Add substrate: add the TMB substrate into each well, 100ul per well. Cover the plate again and then incubate at room temperature again for 10min. Color reaction will occur in the plate.

12) Stop the reaction: add 50ul stop solution into each well, the color will turn yellow from blue.

13) Read the plate: using ELISA reader to read the plate at 450nm. If possible, 630nm can be used as reference wavelength.

## 10. Result Determination

The test results are valid only if the Average OD value of negative control is below 0.250 and the Average OD of positive control is greater than 0.500. Otherwise, please run the analysis again with new kit.

1) Calculation of S/P:

$$\frac{\text{Mean OD of Sample} - \text{Mean OD of Negative Control}}{\text{Mean OD of Positive Control} - \text{Mean OD of Negative Control}} = \text{S/P}$$

2) Criteria of Positive and Negative results.

**Positive: S/P ≥ 0.2**

**Negative: S/P < 0.2**

## 11. Performance of Test

According to field test with over 500 samples, the sensitivity of the kit is 96.8%, and the specificity of the kit is 99.7%.

## 12. Storage and expiration

The kit shall be store at 2-8oC, avoid direct sunlight.

The valid period is 12 months.

## 13. References

(1) [https://en.wikipedia.org/wiki/Avian\\_sarcoma\\_leukosis\\_virus](https://en.wikipedia.org/wiki/Avian_sarcoma_leukosis_virus)

(2) Yun B, Li D, Zhu H, et al. Development of an antigen-capture ELISA for the detection of avian leukosis virus p27 antigen.[J]. Journal of Virological Methods, 2013, 187(2):278-283.