Data Sheet

Avian Leukosis Virus P27 Antigen(ALV-P27) ELISA Kit

Catalogue No.:CK-E10010

Specification: 96T;96T*2;96T*5

Storage Conditions: The kit shall be stored at 2-8 ℃. Avoid moisture.
Shelf life: 12 months. Please use within 2 months after opening. The date of manufacture is presented in the label of the box.

Sample: egg white, meconium, tissue samples, and cell cultures of chickens

Intended Use:

Avian leukosis is a poultry disease characterized by tumors in hemopoietic tissues, including lymphoid, myeloid, and erythroid leucosis. The p27 protein is a key structural component of avian leukosis virus (ALV), comprising 30% of its total protein. Its amino acid sequence is highly conserved and contains group-specific antigens of retroviruses, making it easily detectable. Due to the simplicity and convenience of ELISA kits, they are widely used for rapid screening in production practices.

This kit is used to detect avian leukosis virus p27 antigen in egg white, meconium, tissue samples, and cell cultures of chickens.

Instrument:

Equipment: microplate reader, adjustable micropipette, constant temperature device (25°C), centrifuge, grinder (for tissue samples only).

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Components:

Reagent	Specification		
Microtiter Plate	96wells	96wells×2	96wells×5
HRP conjugate (red cap)	1×11mL	2×11mL	2×26mL
Concentrated Wash Buffer (20×) (white cap)	1×40mL	1×40mL	1×200mL
TMB substrate (black cap)	1×10mL	2×10mL	2×25mL
Stop Solution (yellow cap)	1×5mL	1×10mL	1×25mL
Positive Control (red cap)	1×1.0mL	1×1.5mL	1×2.0mL
Negative Control (green cap)	1×1.0mL	1×1.5mL	1×2.0mL
Adhesive Membrane	1	2	5
Sealed bag	1	1	2
Instructions	1	1	1

Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

1. **Solution preparation**: Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19). What obtained is the **working wash buffer.**
2. **Sample Preparation**:

   Egg white: Collect the fresh egg white and use it directly as a sample for detection without dilution. If the egg white is too viscous, it can be repeatedly frozen and thawed twice to reduce its viscosity.

   Meconium: Place the fresh meconium into a centrifuge tube containing 0.5 mL of working wash buffer and freeze-thaw it twice. After returning to room temperature, centrifuge at 5000 rpm for 2 minutes and collect the supernatant for detection.

   Tissue sample: Take 0.5 g of fresh tissue and place it in a grinding tool. Add 0.5 mL of working wash buffer and grind into a homogenate. Transfer the tissue suspension to a centrifuge tube, freeze-thaw it twice, and centrifuge at 5000 rpm for 2 minutes. Collect the supernatant for detection.

   Cell cultures: Freeze-thaw the fresh cell culture twice. After returning to room temperature, centrifuge at 5000 rpm for 2 minutes (if using a cell culture plate, let it stand for 30 minutes). Collect the supernatant for detection.

   The negative and positive controls should be used directly without dilution.

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells

into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

1. Put the required number of the wells on the plate and set up 2 wells each for negative/positive control.
2. Add 100μL of **negative control** to each negative control well. Then add 100μL of **positive control** to each positive control well. For each sample well, add 100μL of treated sample.
3. Shake gently by hand (or use a microplate shaker) for 5s, cover with adhesive membrane and incubate at 20-25°C in the dark for 60 minutes.
4. Discard the liquid from the wells. Add 300μL of **working wash buffer** to each well, no need to let it stand, then discard. Repeat the washing process 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).
5. Add 100μL of **HRP conjugate** to each well, cover with adhesive membrane, and incubate at 20-25°C in the dark for 60 minutes.
6. Washing. Same as step **4**.
7. Add 100μL of **TMB substrate.** shake gently by hand (or use a microplate shaker) for 5s, cover with adhesive membrane, and incubate at 20-25°C in the dark for 15 minutes.
8. Add 50μL of **stop solution** to each well and shake gently by hand (or use a microplate shaker) for 5s. Read absorbance (**Optical Density; OD**) at 630nm with microplate reader. Finish this step within 10min.

◆ Reference Value

Under normal experimental conditions，the average OD value of the negative control(ODNC), should be <0.2, while the average OD value of the positive control(ODPC), should be ⩾0.5.

◆ Interpretation of Test Results

1. S/P＝ $\frac{OD_S}{OD_{PC}}$

OD_S—the OD value of the sample;

OD_{PC}— the average OD value of the positive control

If **S/P** is > 0.2, it is considered positive; if **S/P** is ≤0.2, it is considered negative.

◆ Limitations of the Test Method

This test is only for the qualitative detection of ALV P27 antigens.

Attention

- During the experiment, gloves and lab coats should be worn. Strict and comprehensive disinfection and isolation protocols should be followed. All experimental waste should be treated as infectious material.
- The stop solution is corrosive. Avoid contact with skin and clothing. If accidentally contacted, rinse immediately with a large amount of tap water.
- **When taking the microtiter plate out of a refrigerated environment, it should be brought to room temperature before opening the bag.** Unused microplate wells should be stored in the sealed bag with a desiccant.
- During washing, each well should be filled completely with liquid to prevent any residual enzyme on the well's rim from remaining unwashed.
- The samples used for testing should be kept fresh.
- The determination of test results must be based on the readings from the microplate reader.
- Components from different lot numbers must not be mixed.