Aleutian Mink Disease Virus Antibody ELISA Kit

AMDV Ab Test

Product Number: E10261
Product Unit: 96T

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1. Introduction

Aleutian disease (AD) is a parvovirus infection characterized by poor reproduction, gradual weight loss, oral and GI bleeding, renal failure and uremia, and high mortality. All color phases of mink may be infected, but light color phases genetically derived from the Aleutian color phase are most susceptible. The causative parvovirus is not related to mink viral enteritis (see Mink Viral Enteritis). Transmission occurs in utero and by direct or indirect contact with infected mink.

In North Europe, eradication program was started to eliminate AMDV from mink ranches, and great effect was observed. In US, Canada and other fur exporting countries, AMDV was also being eradicated.

Generally, AD is controlled through a test and slaughter program. Positive mink are identified by blood testing for specific antibody by counterimmunoelectrophoresis or lateral flow ELISA. All positive mink should be culled. Mink to be kept for breeding stock should be tested in late fall (before selection of breeding stock and pelting) and in January or February (before breeding). New introductions to the herd should be tested.

2. Description of Test

The current ADV-Ab ELISA kit is designed to detect ADV specific antibody induced by ADV infection in mink and ferret. The 96well microtiter plate was precoated with recombinant ADV protein. During testing, samples are added into the microplate wells, in which the precoated antigen will capture the ADV antibody in sample and formed antigen-antibody complex. None specific antibody are discarded by a washing step. Then HRP conjugated secondary antibody is added into each well. After another washing step to remove unreacted conjugate, substrate is added and a blue color will be developed if ADV antibody is present. The enzyme reaction is stopped and the OD450nm value is measured. The measured intensity is positively proportional to the amount of antibody present in the sample.

This ELISA kit can be used to detect ADV specific antibody level in serum / blood.

3. Precautions

- Store the kit at 2-8℃,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

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1	Microplate pre-coated with recombinant protein	1plate, 96T
2	Positive Control	1ml
3	Negative Control	1ml
4	Enzyme conjugate	7ml
5	TMB substrate	7ml
6	**Stop solution**	**Not provided**
7	25X Wash buffer	30ml
8	Kit instruction	1set

6. Instrument Required

- ELISA reader with 450nm/630nm(optional)
- Micropipette 20-200ul, 100-1000ul
- Micropipette Multi-Channel 50-300ul

7. Reagent Preparation

- **Stop solution**: 2M sulfuric acid, please prepare in your lab.
- **Wash buffer (sample 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

- **Serum:** dilute serum with sample buffer in the volume ratio of 1:100, for example, 5ul serum + 500ul sample buffer, mix thoroughly.
- **Blood comb: IMPORTANT**
  (1) **Collect mink blood** with blood paper provided in the kit, record the sample number on the blood paper. When the blood paper is dry, keep it in zip-bag for future use.
  (2) **Attach all the blood paper** to the blood comb. Dip it into the microwells with sample buffer when running the assay. (see attached schematic diagram for direction)

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) **For blood comb sample**: please add 100ul sample buffer into the microwells, and then dip the **blood comb into the wells**, make sure the blood end is dipped **in the solution**. Incubate at 37°C for 10min. After the incubation, discard the blood comb, and the solution in the well will be the sample solution for assay.
For serum sample: add 50ul diluted serum sample into the wells.
4) **Add Positive** control: add 50ul positive control into the wells.
5) **Add Negative** control: add 50ul negative control into the wells.
7) **Incubation**: cover the plate with plate cover and incubate at **37°C for 20min.**
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.

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9) **Add enzyme conjugate**: add 50ul of enzyme conjugate into each well. Cover the plate again and then incubate at **37°C for 20min.**

10) Washing: repeat the washing step again.

11) **Add substrate**: add the TMB substrate into each well, 50ul 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

1) Calculation of S/P:

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

2) Criteria of Positive and Negative results.

Positive: **S/P ≥ 0.2**

Weak Positive: 0.15 < S/P < 0.2

Negative: **S/P ≤ 0.15**

11. Performance of Test

According to field test with over 320 samples, the sensitivity of the kit is 94.3%, and the specificity of the kit is 97.2%.

12. Storage and expiration

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

The valid period is 12 months.

13. References

(1) Viral Diseases of Mink, by Hugh Hildebrandt, DVM, Medford Veterinary Clinic, http://www.msdvetmanual.com/exotic-and-laboratory-animals/mink/viral-diseases-of-mink

(2) Andersson, A.M., A.K. Nyman, and P. Wallgren. 2016. Serodiagnosis of Aleutian disease virus infection in mink – Short term stability and long term consistency of antibody levels measured by VP2 ELISA. Veterinary Sciences: Research and Reviews, 2(1): 23-30.

(3) Ma F, Zhang L, Wang Y, et al. Development of a Peptide ELISA for the Diagnosis of Aleutian Mink Disease. Hegde NR, ed. PLoS ONE. 2016; 11(11):e0165793. doi:10.1371/journal.pone.0165793.

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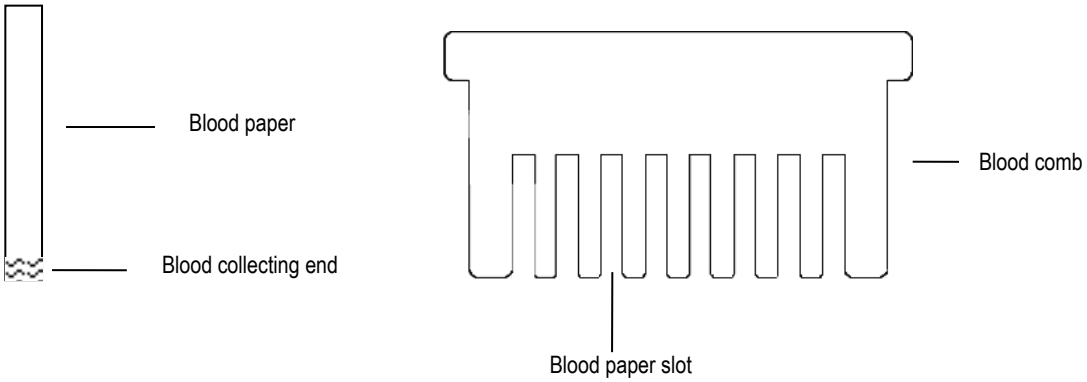
How to use the blood comb and blood paper

1. Check the kit content, identify the following components

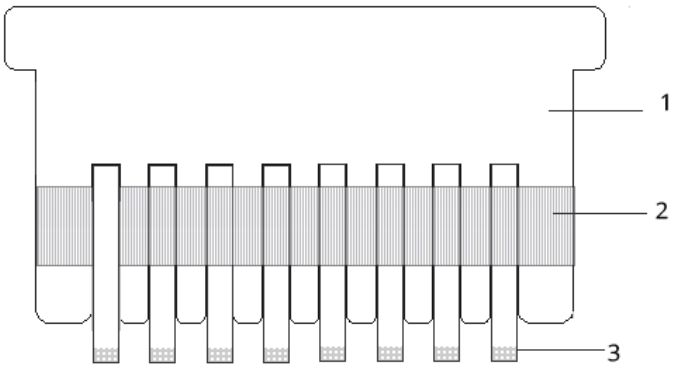
Blood Paper: for blood sample collecting. Bleed the mink, and collect the blood with the blood end of the blood paper. Make sample number record on the blood paper.

Blood comb: for blood paper supporting. To provide a support when running the assay. Attach the collected blood paper in the slot of the comb, and stick on it with adhesive tape.

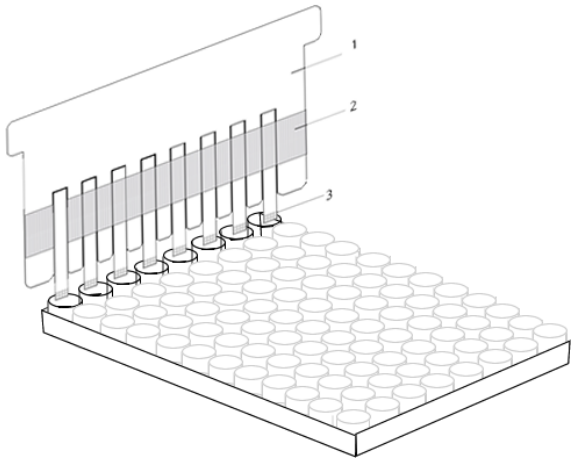


2. Attach the blood paper to the blood comb

In the picture below, No. 1 is the Blood Comb. No 2 is the adhesive tape. No 3 is the blood paper.



3. **Release the sample**: take out the 96well plate and add 100ul sample buffer in into the microwells. Insert the prepared blood comb in well and incubate at 37°C.



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