Actinobacillus pleuropneumoniae Antibody Test Kit
Swinecheck MP® APP 1-9-11, 2, 3-6-8-15, 4-7, and 5
Insert

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Swinecheck MP® APP assay is a mutiplex immunoassay intended for determining the presence of antibodies to ***Actinobacillus pleuropneumoniae*** **(APP)** serogroups **1-9-11, 2, 3-6-8-15, 4-7, and 5** in swine serum.

PRINCIPLE OF THE ASSAY

Swinecheck MP® APP assay is a microsphere-based multiplex fluorescent immunoassay (MFIA)-type antibody detection test. A total of 5 distinct microsphere sets (coated with either APP 1-9-11, APP 2, APP 3-6-8-15, APP 4-7, or APP 5 antigens) are incubated in diluted swine serum and then washed. Successive incubations with biotinylated anti-swine IgG (Detection Antibody) and streptavidin R-phycoerythrin (SA-PE) reporter follow, each succeeded by a wash step to remove unbound reagent. Users place plates in the plate analyzer, which captures the fluorescence intensity of the microspheres and SA-PE reporter. An S/P ratio of the SA-PE on the antigen-coated microspheres above the provided cut-offs indicates antibody is present in the sample.

MATERIAL

Components Supplied in the Kit

Component	Quantity	Storage
Sample Diluent	150 mL	2 to 7°C
APP 1-9-11 Positive Control (ready-to-use)	2 mL	2 to 7°C
APP 2 Positive Control (ready-to-use)	2 mL	2 to 7°C
APP 3-6-8-15 Positive Control (ready-to-use)	2 mL	2 to 7°C
APP 4-7 Positive Control (ready-to-use)	2 mL	2 to 7°C
APP 5 Positive Control (ready-to-use)	2 mL	2 to 7°C
Negative Control (ready-to-use)	3 mL	2 to 7°C
Microsphere Mix	28 mL	2 to 7°C
Wash Buffer (10X)	500 mL	2 to 7°C
Detection Antibody	54 mL	2 to 7°C
SA-PE Conjugate	54 mL	2 to 7°C
Microtiter Plate	5	2 to 25°C

The materials provided are sufficient for testing up to 445 samples.

Microsphere Mix Constituent	Microsphere Code (Region)
APP 1-9-11 antigen	51
APP 2 antigen	52
APP 3-6-8-15 antigen	53
APP 4-7 antigen	54
APP 5 antigen	55

Equipment and Consumables Required But Not Provided

- Purified water
- Table-top bath sonicator
- Vortex
- Plate shaker
- Magnetic microsphere separator
- Plate analyzer (e.g. LX200, Magpix)
- Data analysis software (e.g. xPONENT)
- Aluminum foil
- Bottles, tubes or plates for dilution
- Micropipettes and tips
- Microcentrifuge
- Plate washer (**optional**)
- Additional 96-well round bottom white non treated polystyrene microplates (if required)

PRECAUTIONS AND WARNINGS

- Handle all assay materials as if they are capable of infection. Although the bacterial antigens have been chemically inactivated prior to coating on the microspheres, follow proper biological safety procedures while handling the materials and wear appropriate personal protective equipment.
- All wastes should be properly decontaminated prior to disposal.
- Kit components contain ProClin® to prevent microbial growth. This can cause allergic reactions in some people. The ProClin® content is <0.05%.
- The magnetic field generated by the magnetic separator interferes with electrical medical devices.
- **Microsphere Mix, Detection Antibody, and SA-PE Conjugate are light sensitive**. **Store them protected from light.**
- Store all materials as indicated in the Components Supplied in Kit table, above, and on the labels of the kit components.
- Reagents should be stored back at appropriate temperature as soon as possible after use.
- Do not use reagents beyond their expiration dates and do not use them with components from different serials.
- To achieve reliable results, follow proper laboratory technique and pipette accurately. Take care to mix dilutions properly and use correct pipetting technique.
- This assay is for *in vitro* veterinary use only.

TECHNICAL SUPPORT

For technical support, send inquiries by e-mail to support@biovet-inc.com

ASSAY EXECUTION

Prepare the Samples and Reagents

1. Allow reagents to equilibrate to room temperature (19-26°C) for at least 30 minutes before proceeding (the Sample Diluent has to become perfectly liquid).
2. Dilute the samples 1/100 in Sample Diluent and mix thoroughly.
3. After homogenizing the 10X Wash Buffer (no evidence of crystals), dilute 1/10 with purified water. The Wash Buffer 1X solution is stable for 1 week at 2-7°C.
4. Vortex the Microsphere Mix for approximately 5 seconds, then sonicate using a bath sonicator (20 to 35 W, 40 to 60 kHz) for approximately 5 seconds.

Prepare the Plate with Diluted Samples and Controls

5. Make a schematic representation of the plate and the distribution of controls and samples.
6. Dispense 50 µL of Microsphere Mix to each well of the sample plate that will contain samples or controls.
7. Dispense 50 µL of the ready-to-use Negative Control to wells A1 and B1.
8. Dispense 50 µL of the ready-to-use APP 1-9-11, APP 2, APP 3-6-8-15, APP 4-7, and APP 5 Positive Controls respectively to wells C1, D1, E1, F1, and G1.
9. Dispense 50 µL of diluted sample to the appropriate wells of the plate.
10. Cover the entire plate with aluminum foil or an equivalent opaque material to protect it from light.
11. Place the plate on a plate shaker and incubate at room temperature (19 to 26°C) for 60 minutes at 800 rpm.
12. Move the plate to a 96-well magnetic separator for a minimum of 1 minute.
13. While leaving the plate on the magnetic separator, discard the supernatant, ensuring that there is no disruption to the microspheres.
14. Do not remove the plate from the magnetic separator and dispense 100 µL of 1X Wash Buffer into each well, incubate for one minute, then discard the Wash Buffer as in step 13.
15. Repeat step 14 to perform another wash cycle (for a total of 2 wash cycles), remove the plate from the magnetic separator.

Add the Detection Antibody

16. Dispense 100 µL of the Detection Antibody to each well of the plate.
17. Cover the entire plate with aluminum foil or an equivalent opaque material to protect it from light.
18. Place the plate on the plate shaker and incubate at room temperature (19 to 26°C) for 30 minutes at 800 rpm.
19. Discard the supernatant and perform 2 wash cycles as described in steps 12 through 15.

Add the SA-PE Conjugate

20. Dispense 100 µL of SA-PE Conjugate to each well of the plate.
21. Cover the entire plate with aluminum foil or an equivalent opaque material to protect it from light.
22. Place the plate on the plate shaker and incubate at room temperature (19 to 26°C) for 30 minutes at 800 rpm.
23. Discard the supernatant and perform 2 wash cycles as described in steps 12 through 15.

Analyse the Plate

24. Dispense 125 µL of 1X Wash Buffer into each well and resuspend the microspheres using the plate shaker (2 min at 800 rpm).
25. Load the plate into the plate analyzer and run the batch.

RESULT VALIDITY AND INTERPRETATION

Result Validity

Assay results must meet the following criteria to be valid:

1. Each sample requires the analysis of at least 25 microspheres per antigen for valid data. Rerun any sample with a lower microsphere count for any antigen.
2. The mean "MFI" (MFI: median fluorescence intensity) signal for the Negative Control must be <1,000. Higher Negative Control signals can indicate a systematic error for the assay plate and require repeating the assay plate.
3. The "corrected MFI" signal for the Positive Controls must be ≥4,000 for all controls. Lower Positive Control signals can indicate a systematic error for the assay plate and require repeating the assay plate.

Data Analysis

The S/P ratio is intended to differentiate positive from negative specimens. Calculate the S/P ratio for each sample using the formulas in the Calculations section.

	negative	suspicious	positive
APP 1-9-11	S/P < 0.40	0.40 ≤ S/P < 0.55	S/P ≥ 0.55
APP 2	S/P < 0.30	0.30 ≤ S/P < 0.40	S/P ≥ 0.40
APP 3-6-8-15	S/P < 0.30	0.30 ≤ S/P < 0.40	S/P ≥ 0.40
APP 4-7	S/P < 0.40	0.40 ≤ S/P < 0.50	S/P ≥ 0.50
APP 5	S/P < 0.30	0.30 ≤ S/P < 0.40	S/P ≥ 0.40

Calculations

1. Calculate the Negative Control mean MFI for each antigen:
   - E.g. [(Well A1 MFI + Well B1 MFI)/2] for each of the APP 1-9-11, APP 2, APP 3-6-8-15, APP 4-7, and APP 5 antigens
2. Calculate the "corrected MFI" for each of the Positive Controls with its corresponding antigen:
   - for APP 1-9-11: Well C1 MFI – Negative Control mean MFI
3. For each sample and each antigen, calculate the sample "corrected MFI": sample MFI - Negative Control mean MFI
   - E.g. for sample in well H1 and APP 1-9-11: Well H1 MFI - Negative Control mean MFI for APP 1
4. For each sample and each antigen, calculate the S/P Ratio = Sample "corrected MFI" / Positive Control "corrected MFI"

Example for APP 1-9-11

Negative Control (wells A1 and B1) :

- MFI = 210 and 190
- Negative Control mean MFI = [(210 + 190)/2] = 200

APP 1 Positive Control (well C1) :

- MFI = 5,000
- Corrected MFI = (5,000 – 200) = 4,800

Sample #1 (well H1)

- MFI = 6,000
- Corrected MFI = (6,000 – 200) = 5,800
- S/P ratio = 5,800/4,800 = 1.21 (positive)

Sample #2 (well A2)

- MFI = 300
- Corrected MFI = (300 – 200) = 100
- S/P ratio = 100/4,800 = 0.02 (negative)