

GENERAL DESCRIPTION

The HRPro® MS AB ELISA is designed to detect presence of antibodies against Mycoplasma Synoviae(MS) in chicken serum. It provides a rapid, simple and specific method for detecting antibodies against MS and has highly correlation with Hemagglutination-inhibition (HI) antibody test.

The HRPro® MS AB ELISA is performed in plate that is coated with purified inactivated mycoplasma antigen. If anti-MS antibodies present in the sample, color will develop after addition of HRPO (horseradish peroxidase) conjugated Anti-Chicken antibody and the chromogen-containing substrate. The OD (optical density) of color development is proportional to the amount of antibody specific to MS in the chicken sera. The log titer of antibody in the chicken serum is obtained by calculated formula. It shows high correlation rate with HI titer in MS infected chickens.

KIT COMPONENTS

Reagents	480 tests
① MS Antigen Coated plate	5 plates
② 10X Washing Buffer	240ml X 1
③ Dilution Buffer	240ml X 1
④ HRPO Anti-Chicken IgG Conjugate	70ml X 1
⑤ Positive Control, PC	7.0ml X 1
⑥ Negative Control, NC	7.0ml X 1
⑦ TMB Substrate	70ml X 1
⑧ Stop Solution	40ml X 1
⑨ Sealing Film	5 sheets
⑩ Instruction Manual	1 copy

PREPARATION

- All reagents must be allowed to come to room temperature (20~25°C) before use. Mix reagents by gentle swirling. After use, return to 2~8°C.
- 1X washing buffer preparation
 - Shake 10X Washing Buffer(②) gently.
 - Dilute 1 part of 10X Washing Buffer(②) with 9 parts of deionized water. The diluted 1X washing buffer is stable for 2 weeks at room temperature (20~25°C).
- Sample preparation
 - Use the serum samples as fresh as possible
 - Use fresh samples for the best result. Serum samples can be stored at 2~8°C for less than 3 days or -20°C for a longer period. Do not freeze and thaw serum samples repeatedly. **Sera with hemolysis or bacterial contamination are not suitable for the analysis!**
 - Visible solid materials in serum samples should be separated by centrifugation.

- Sample dilution
 - Prepare the 1 ml deep-well-plate (DWP, 96-well, not offered) or suitable tubes.
 - Please choose the appropriate serum dilution method.
 - When you examine several virus simultaneously dispense 1000 of Dilution Buffer(③) into well of deep well plate (96 well) or microtube and add 2 of test samples (1:500 dilution)
 - When you examine only MS, dispense 500 of Dilution Buffer(③) into well of deep well plate (96 well) or microtube and add 1 of test samples (1:500 dilution)
- Diluted Samples should be mixed prior to dispensing into the Antigen Coated Plate(①).
- Do not dilute the positive control(PC, ⑤) and negative control(NC, ⑥).**
- TMB Substrate (⑦) should be warmed up for 30 minutes at room temperature (20~25°C) before use (10ml/plate). If stored at low temperature, the color development may be poor.

TEST PROCEDURE

- Remove the Antigen Coated Plate (①) from protective foil pouch.
- Dispense 100 of diluted sample into appropriate wells. **Use care not to spill samples from well to well.**
- Dispense 100 of undiluted NC(⑥) and undiluted PC(⑤) into triplicate wells.
- Seal the plate and Incubate for 30 minutes at 25 ± 1.0 °C.
- Wash each well 3 times with 1X washing buffer (300 per well). And remove contents of well at each washing steps. After final washing, hit plate to the paper towel briefly to remove solution.
- Dispense 100 of HRPO Anti-Chicken IgG Conjugate(④) to each well.
- Seal the plate and incubate for 30 minutes at 25 ± 1.0 °C.
- Wash each well as step 5.
- Dispense 100 of TMB substrate (⑦) to each well.
- Seal the plate and incubate for 15 minutes at 25 ± 1.0 °C. Check the density of color development by naked eyes.
- Add 50 of Stop Solution (⑧) to each well of the plate. Shake the test plate shortly (5~10 sec). **Be careful not to spill.**
- Measure and record the A (450nm) for samples and controls immediately.
- Validate and calculate the results.

Plate template example (1-well Test)

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	PC	19	27	35	43	51	59	67	75	83
B	NC	NC	NC	20	28	36	44	52	60	68	76	84
C	1	7	13	21	29	37	45	53	61	69	77	85
D	2	8	14	22	30	38	46	54	62	70	78	86
E	3	9	15	23	31	39	47	55	63	71	79	87
F	4	10	16	24	32	40	48	56	64	72	80	88
G	5	11	17	25	33	41	49	57	65	73	81	89
H	6	12	18	26	34	42	50	58	66	74	82	90

RESULT INTERPRETATION

1. Validate if the mean OD of the PC (PCx) is higher than 0.35 and the mean OD of the NC (NCx) is lower than 0.20.
If these criteria are not met, the test are invalid and the samples must be retested.
2. Calculate the sample to positive ratio (S/P ratio) by following the formula

$$\text{S/P ratio} = \frac{\text{Sample OD} - \text{NC mean OD}}{\text{PC mean OD} - \text{NC mean OD}}$$

Calculate the Log titer.

$$\text{Log Titer} = 1.2 (\log \text{S/P}) + 3.20$$

3. Result interpretation

- 1) Test samples having ≥ 0.3 S/P ratio are positive.
- 2) Test samples having < 0.3 S/P ratio negative.

S/P value	Interpretation
$\text{S/P} \geq 0.5$	Positive
$\text{S/P} < 0.5$	Negative

PRECAUTIONS

1. All reagents must be allowed to come to room temperature (20~25°C) before use. Mix reagents by gentle swirling. After use, return to 2~8°C.
2. Read this instruction manual thoroughly and follow all steps strictly for successful use of the product.
3. All test samples should be considered potentially infectious and all items contacting the samples should be considered contaminated.
4. Do not use expired or contaminated reagents.
5. Do not use reagents from other kits or lots.
6. Do not mix reagents from different lots of this same product.
7. Do not expose the reagents to excessive heat or direct light during storage and incubation.
8. Incomplete washing adversely may affect the result and precision of the assay.
9. Avoid microbial contamination of the reagents.
10. Avoid contamination of the TMB Substrate(7) with the HRPO Anti-Chicken IgG Conjugate(4).
11. Wear personal protective equipment (PPE) such as lab coat, goggles, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
12. Do not eat, drink, smoke or apply cosmetics where kit reagents are handled. Do not pipette by mouth.
13. Pipette tips must be changed after each pipetting step. Use a clean disposable pipette tip for all steps.
14. Use care not to spill samples from well to well.
15. Deionized water or equal must be used to prepare the washing buffer.
16. Unused strips should be stored in the sealed foil pouch at 2~8°C. Re-sealed strips are recommended to use within one week.
17. For veterinary use only.

STORAGE AND STABILITY

Store all reagents at 2~8°C. Do not freeze. Reagents remain stable until the expiration date when stored as instructed. 

Manufactured by VETIPEX Inc.

10665 Jasper Avenue, 14th Floor Edmonton, Alberta, T5J 3S9, Canada.

Tel: 001 780 604 7810

E-mail: info@vetipex.com

www.vetipex.com

QUICK PROTOCOL

Serum dilution 1/500



MS Antigen Coated Plate



Add 100 μl of diluted Samples
&
Add 100 μl of Undiluted PC, NC



RT, 30min



Washing 3 times

Dispense 100 μl of
HRPO Anti -Chicken IgG Conjugate



RT, 30min



Washing 3 times

Dispense 100 μl of
TMB Substrate



RT, 15min



Dispense 50 μl of
Stop Solution



Measure OD at 450nm