

SERELISA[®] M.hyp Ab Mono Blocking

KIT FOR THE DETECTION OF ANTI-MYCOPLASMA HYOPNEUMONIAE ANTIBODIES IN SWINE SERUM OR PLASMA (INDIVIDUAL)

BLOCKING IMMUNOENZYMATIC TECHNIQUE

384 single well reactions

I. PRINCIPLE OF THE TEST

The SERELISA[®] M.hyp Ab Mono Blocking kit uses a single well blocking immunoenzymatic technique for the detection of antibodies to a membrane protein of *Mycoplasma hyopneumoniae*. There are three steps:

1. Each serum or plasma sample is placed in a well sensitised with the *Mycoplasma hyopneumoniae* membrane protein. Antibodies present in the sample bind specifically onto the antigen coated to the wells.

2. An anti-protein monoclonal antibody (MAb) peroxidase conjugate is added. This monoclonal antibody which binds to a *Mycoplasma hyopneumoniae* specific epitope fixes onto the free antigenic sites forming a complex :

(Ag) - (MAb anti-protein / peroxidase).

3. Excess conjugate is eliminated by a wash step. The enzyme linked to the complex is revealed by the addition of a substrate which transforms it into a coloured product. The corresponding optical densities are read and interpreted as follows:

- In the absence of antibodies in the sample, an intense coloured reaction is observed due to the transformation of the enzyme conjugate bound to the free membrane protein sites fixed to the solid support.
- In the presence of *Mycoplasma hyopneumoniae* antibodies in the sample, less enzyme conjugate is bound to the antigenic sites on the solid support and thus the coloured reaction is diminished.

II. KIT COMPOSITION AND CONSERVATION

REAGENT NATURE	RECONSTITUTION AND CONSERVATION
4 microplates containing 6 strips of 16 wells sensitised with a <i>M. hyopneumoniae</i> membrane protein.	Use within 1 month after opening of the sachet which must be closed after use.
Wash solution (W) (10X concentrated)	Dilute 10 times in distilled or demineralised water. Use within 5 days after dilution.
Sample diluent pink color (SD). May contain harmless precipitate.	Ready-to-use.
Negative control (N)	Ready-to-use.
Positive control (P)	Ready-to-use.
Conjugate diluent blue color (CD)	Ready-to-use.
Conjugate (concentrated 100 times) Mab anti-protein / peroxidase (CJ)	To be diluted 100 times in the CD. Use within 4 hours after dilution.
Buffered peroxidase substrate (PS)	Ready-to-use.
Stop solution (S)	Ready-to-use.
Adhesive films	12 films

Note: Kit and diluted reagents should be stored at + 5°C ± 3°C and used as mentioned above.

Reference : SHYO1N.NA version n2 – 25/06/09

Version n1 → n2 : nomenclature and trademark modifications

III. MATERIALS AND REAGENTS REQUIRED (NOT SUPPLIED)

- Distilled or demineralised water.
- Adjustable or set pipettes to measure and deliver between 0 to 1000 µl. Measurement deviation must be ≤ 10% for volumes ≤ 10 µl and ≤ 5% for all other volumes.
- Graduated cylinders (100 ml and 1000 ml).
- Manual, automatic or semi-automatic washing device for microtitration plates.
- Microplate reader, fitted with filters for bichromatic reading at 450 and 630 nm. It is also possible to use a monochromatic reader fitted with a 450 nm filter.

IV. PRECAUTIONS FOR USE

The quality of the results depends on the respect of good laboratory practices and the procedure (see paragraph VI).

1. Do not mix or associate reagents from kits with different batch numbers.
2. Do not use reagents after the expiry date.
3. Place all reagents at laboratory temperature for at least 1 hour prior to use.
4. Handle all reagents and samples as biohazardous material.
5. Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
6. Never pipette by mouth.
7. Avoid inter sample contamination during sample collection, storage or transport. Use separate disposable pipette tips for each sample.
8. Avoid contamination of the substrate solution with metallic ions, oxidizing agents or detergents. Make sure that all containers are clean. Do not use the same container or the same pipette tip for the conjugate and the substrate.
9. It is recommended to dispose reagents and contaminated material according to the applicable regulations. The safety data sheets for the product are available upon request.

Risk phrases:

- R 23/25: Toxic by inhalation and if swallowed.
R35: Causes severe burns.
R 36/37/38: Irritating to eyes, respiratory system and skin.
R 41: Risk of serious damage to eyes.
R 42/43: May cause sensitisation by inhalation and skin contact.
S 7: Keep container tightly closed.
S 24: Avoid contact with skin.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S30: Never add water to the product.
S45: In case of accident or if you feel unwell, seek medical advice immediately.

V. SAMPLES

The test is performed on serum or plasma samples diluted 1:2.

Samples should be stored as follows:

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (20°C)
Plasma or serum.	max. 7 days	Yes	No

VI. PROCEDURE

Strictly comply with the procedure indicated below. Use negative and positive controls in duplicate for each test run, for each plate.

A. PRELIMINARY STEPS

1. Carefully set up the distribution and identification of controls and samples.
2. Prepare the serum or plasma samples to be tested. Dilutions can be performed either beforehand in dilution plate, or directly in the test wells.

B. TEST PROCEDURE

I - CONTROL AND SAMPLE DISTRIBUTION

1. Control distribution:

Controls are ready-to-use.

After shaking the vials, add 100 µl of negative control (N) to wells A1 and A2, and 100 µl of positive control (P) to wells B1 and B2.

2. Sample distribution:

Place 100 µl of the 2-fold diluted samples per well.

For diluting directly in the wells, place 50 µl of sample diluent (SD) plus 50 µl of serum in the well, and mix thoroughly.

Samples can be tested individually or in duplicate.

Strips should always be placed on the frame so that both washer and reader can be used.

Cover the wells with adhesive film, cut to the necessary length by the number of strips used.

Mix by gentle shaking the plate manually or by using a plate agitator.

3. Incubation of the plate:

Incubate the plate during 45 minutes ± 2 min at + 20°C ± 5°C.

**!!! DO NOT WASH AFTER INCUBATION
DO NOT REMOVE LIQUID**

II – ADDITION OF CONJUGATE

1. Preparation of conjugate:

Prepare the conjugate solution by diluting the concentrate (CJ) 1:100 in the conjugate diluent (CD); 1 ml for one strip, meaning 10 µl of CJ in 0.99 ml of CD.

2. Distribution of conjugate:

Add 100 µl of diluted conjugate to all the wells and cover with a new piece of adhesive film.

Mix by gentle shaking the plate manually or by using a plate agitator.

3. Incubation of conjugate:

Incubate for 30 minutes ± 2 min at + 20°C ± 5°C.

WASHING:

Wash buffer: dilute the concentrated washing solution (W) 1:10 in distilled or demineralised water.

Carefully remove the adhesive film and wash 4 times.

III – REVELATION

1. Addition of the substrate:

Add 100 µl of peroxidase buffered substrate (PS) per well. Do not cover with adhesive film at this stage. Mix by gentle shaking the plate manually or by using a plate agitator to ensure correct mixing.

2. Incubation of substrate:

Incubate for 15 minutes ± 2 min at laboratory temperature (+ 20°C ± 5°C), shielded from light.

3. Addition of Stop Solution:

Add 50 µl of stop solution (S) per well.

Mix by gentle shaking the plate manually or by using a plate agitator. Make sure that no bubbles occur in the wells.

4. Measure of the optical density:

Measure the optical density (OD) bichromatically at 450 and 630 nm or monochromatically at 450 nm (in the yellow band).

Reading bichromatically is strongly recommended. Should a monochromatic reader be used, ensure the cleanliness of the bottom of the wells prior to reading.

VII. TEST VALIDATION

Valid SERELISA® M.hyp Ab Mono Blocking result is obtained when:

- The average optical density (OD) value of the Negative controls (A1, A2) is > 0.7.
- The P/N of the average optical density (OD) value of the Positive controls (B1, B2) is less than 0.5

This P/N is calculated as follows:

$$P/N = \frac{\overline{OD} P}{\overline{OD} N}$$

\overline{OD} = average of the optical densities of the negative and positive control.

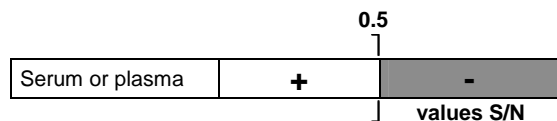
VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

- Divide the sample OD by the average Negative Control OD for the ratio Sample/Negative (S/N).
- S/N of greater than 0.5 is negative and less or equal than 0.5 is positive.

This S/N is calculated as follows:

$$S/N = \frac{\overline{OD} \text{ reading for Sample Absorbance}}{\overline{OD} N}$$

RESULTS



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