HANDBOOK

BioLisa® kit MAP Ab

ELISA kit for the detection of antibodies to Mycobacterium avium paratuberculosis

Method	Matrix	Species	Protocole
Indirect ELISA	Individual serum	Bovine Ovine Caprine	Short incubation

Information : modifications from previous version are indicated by yellow highlighting

Cat.No. : BIOLK001
BIOLK002

MU/MAP/005/EN
Rev 06/20

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For veterinary use only

• General information

Paratuberculosis is an enteric chronical bacterial disease caused by Mycobacterium avium paratuberculosis (MAP), which can affect multiple ruminants species. The most common symptoms are diarrhea, lost of weight and global weakness of the animal. The mycobacteria fecal sheeding by infected animals leads to an environmental contamination and an importante source of within herds contagion. This shedding appears prior to the first clinical symptoms, which imply a regular herd monitoring for the desease control and diagnostic.

BioLisa® kit MAP Ab is a sensitive and specific ELISA test for the detection of antibodies to MAP, in ruminant individual serum. A correlation with fecal shedding exists for bovine.

• Principle of the test

The plates are coated with an inactivated MAP antigen. During the sample incubation, the anti-MAP antibodies bind to the antigen. After a washing step which removes all the no binded proteins, an HRP conjugated anti-ruminant is added. This conjugate specifically bind to the ruminant antibodies which binded to the well (positive sample). The unbound conjugate is removed by washing, and the substrat solution is added. By contact with the HRP enzyme, the substrat solution becomes blue. After stopping the reaction, the colour turns to yellow.
The optical density (OD) is measured by a spectrophotometer. The coloration in the well indicate the presence of a positive sample.

• Kit content

Component		Quantity	
		Cat.No BIOLK001	Cat.No BIOLK002
1	Test plate. 12 strips of 8 wells per plate	2	5
2	Sample diluent	1 x 30mL	1 x 60mL
3	Negative Control	1 x 0.5mL	1 x 1.2mL
4	Positive Control	1 x 0.5mL	1 x 1.2mL
5	Wash buffer 10X	1 x 125mL	2 x 125mL
6	Conjugate 100X	1 x 0.3mL	1 x 0.6mL
7	Conjugate diluent	1 x 30mL	1 x 60mL
8	TMB Substrate	1 x 30mL	1 x 60mL
9	Stop Solution	1 x 30mL	1 x 60mL
Certificat of analysis			
Adhesive plate covers			

The components must be stored at 5±3°C and are stable until the expiration date indicated on the labels. The unused plate strips must be stored in the sealed bag with a desicate packet at 5±3°C. The test strips can be stored for at least 6 weeks after opening the plate bag.

• Symboles

Contains reagents for <N> plates


Legal manufacturer

Lot number


Use by date


Temperature limitations for storage

Catalog number

Material number


Protect from light


For cattle, ovine and caprine samples

• Not provided material

- Monochannel and multichannels pipettes
- Single-use tips
- Microplate absorbance reader with a 450nm filter
- Distilled or deionised water
- Single-use containers
- Pre-dilution plates

• General precautions

Always wear appropriate personal protective equipment. For more information, please consult the appropriate material safety data sheets (MSDS) (availables on request).

- Carefully read and strictly follow the instruction of use
- Handle all materials according to the Good Laboratory Practice.
- Consider all the reagents and samples as potentially contaminated.
- All unused biological materials should be disposed according to the local, regional and national regulations.
- Do not use reagents beyond expiration date.
- Do not mix components from different kit batches.
- Include positive and negative controls on each plate or test strip series.

• Before starting

All reagents should equilibrate to room temperature (21±4°C) before use.

Wash solution : the wash solution 10X must be 10 fold diluted in distilled/ deionised water. *Example : for one plate, dilute 50mL of wash solution 10X into 450mL of water.*
The diluted wash solution could be stored for one month at 5±3°C.

Conjugate : the conjugate 100X must be 100 fold diluted in the conjugate diluent. *Example : for one plate, dilute 100µL of conjugate 100X into 10mL of diluent.*

The diluted conjugate solution must be used during the day after dilution.

Samples : fresh, refrigerated or frozen individual sera could be used.

• Protocol

1. Sample pre-dilution 1:12 :
   In a pre-dilution plate, add 110µL of sample diluent into each well.
   Add 10µL of positive control (PC) and negative control (NC) into the appropriated wells *(A1B1 and C1D1 for example).*
   Add 10µL of samples into the following wells.

Gently shake the plate and incubate 15±2minutes at room temperature (21±4°C).

2. Sample distribution :
   Transfer 100µL of pre-diluted samples and controls into the test plate.

Gently shake and cover the plate. Incubate 45±5minutes at room temperature (21±4°C).

3. Washing :
   Empty the wells and wash 3 times with 300µL of diluted wash solution. After the last washing step, empty the plate and softly tap it on an absorbant paper to remove all the liquid.

This step could be done manually or with a washing machine.

4. Conjugate
   Add 100µL of diluted conjugate in each well.

Cover the plate and incubate 30±3 minutes at 37±3°C.

5. Washing :
   Repeat the step 3. Washing.

6. Revelation :
   Add 100µL of substrate in each well.

Incubate the plate 10±1min at room temperature (21±4°C) in darkness.

7. Stopping the reaction :
   Stop the reaction by adding 100µL of stop solution in each well following the same order than with the substrate.

8. Reading :
   Measure the optical density (OD) in a microplate reader at 450nm within 30±3min after stopping the reaction. Make sure there is no dust or bubbles into the wells.

- ## Validation criteria

The results are validated if :
- The mean mean OD of the positive control (PC OD) is > 0.6.
- The ratio (mean PC OD / NC OD is > 3.

If one of these criteria is not compliant, the assay should be repeated.

- ## Calculations

Calculate the mean OD for the positive and negative controls.

The percentage of positivity S/P value is calculated for each sample, using the following formula :

$$\% S/P = \frac{\text{Sample OD - Mean NC OD}}{\text{Mean PC OD - Mean NC OD}} \times 100$$

- ## Interpretation of results

Bovine sera

Result	Interpretation
% S/P < 35%	Negative
35% ≤ % S/P < 60%	Low positive +
% S/P ≥ 60%	High positive ++

NB : The two positivity cut-offs were defined after a field study, by correlation with positive predictive values of fecale shedding, corresponding to 50% and 90% respectively.

Ovine and caprine sera
For assay interpretation with ovine and caprine sera, please contact the technical service of Biosellal.