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cattletype® MAP Ab Handbook

For detection of antibodies to *Mycobacterium avium* subsp. *paratuberculosis*

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-B 471

REF 5 plates (cat. no. CT270803)

REF 20 plates (cat. no. CT270805)*

INDICAL BIOSCIENCE GmbH, Deutscher Platz 5b,
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* available only on request

INDICAL BIOSCIENCE

Contents

Kit contents

cattletype MAP Ab **Cat. no.** **Number of plates**	**(5)** **CT270803** **5**	**(20)** **CT270805*** **20**
Test Plate: microtiter plate with 96 wells, coated with non-infectious MAP antigen	5	20
Sample Diluent, ready to use	1 x 100 ml	1 x 400 ml
Negative Control, ready to use	1 x 3.5 ml	2 x 3.5 ml
Positive Control, ready to use	1 x 3.5 ml	2 x 3.5 ml
Wash Buffer, 10x concentrate	3 x 125 ml	2 x 500 ml
Conjugate, ready to use	1 x 60 ml	1 x 240 ml
TMB Substrate, ready to use	1 x 60 ml	1 x 240 ml
Stop Solution, ready to use	1 x 60 ml	1 x 240 ml
Handbook	1	1

* available only on request

Intended use

The cattletype MAP Ab is an indirect ELISA for detecting antibodies to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in serum, plasma, and milk samples from cattle, sheep and goats.

The kit is approved by the Friedrich-Loeffler-Institute and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-B 471) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols

	Legal manufacturer
LOT	Lot number
	Use by date
	Temperature limitations for storage
	Handbook
REF	Catalog number
MAT	Material number
	For samples from cattle, sheep and goats

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of cattletype MAP Ab is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the cattletype MAP Ab ELISA should be stored at 2-8°C and are stable until the expiration date stated on the label. Wash Buffer (10x) and Stop Solution may be stored at room temperature (18-25°C) to avoid salt crystallization. If test strips are provided with the kit, store the remaining test strips in the re-sealed foil pouch with desiccant at 2-8°C until next use. The test strips can be stored for at least 6 weeks after opening the plate pouch.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available from your local sales representative or by Email request under **compliance@indical.com**.

CAUTION: The Stop Solution contains 0.5 M sulfuric acid.

All sample residues and objects that have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Introduction

The cattletype MAP Ab is a highly sensitive solution for the detection of antibodies to *Mycobacterium avium* subsp. *paratuberculosis* (MAP).

MAP is the causative agent for paratuberculosis, which is also called Johne's disease. Paratuberculosis is an incurable and chronic infectious disease with a long incubation time, characterized by excessive weight loss and persistent diarrhea in cattle in the final stage of the disease. MAP is spread worldwide among ruminants. The cattletype MAP Ab Kit permits the semi-quantitative detection of anti-MAP antibodies and can be used with serum, plasma, and milk samples.

Principle

Samples are first diluted and pre-incubated with a Sample Diluent containing inactivated *Mycobacterium phlei* extract in order to minimize cross-reactions to atypical mycobacteria. The microtiter plate is coated with MAP-antigen. During sample incubation MAP-specific antibodies bind to the immobilized antigen. Unbound material is removed by rinsing. The anti- IgG-HRP conjugate detects antibodies bound to the antigen. Unbound conjugate is removed by rinsing. A colorimetric reaction is initiated by adding Substrate Solution and stopped after 10 minutes. In the presence of MAP-specific antibodies, within the sample, HRP catalyzes a blue color development, which turns yellow after adding the Stop Solution. The optical density (OD) is measured in a spectrophotometer. The OD values correlate with the concentration of anti-MAP antibodies in the sample.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Beakers
- Measuring cylinders
- Pipets (adjustable)
- Multichannel pipets (adjustable)
- Aluminum or adhesive foil for covering the Test Plate
- Optional: Device for delivery and aspiration of Wash Buffer
- Microtiter plate absorbance reader
- Tubes or plates for diluting the samples
- Distilled water

Important notes

General precautions

The user should always pay attention to the following:

- Do not expose the TMB Substrate Solution to intense light or to sunlight when performing the test.
- Components of the test kit should not be contaminated or mixed with components from other batches.
- Do not use the components of the test kit past the expiration date.
- Water from ion-exchange systems used for diluting the Wash Buffer (10x) may interfere with the assay if not pure enough. Use double-distilled water or highly purified water (Milli-Q®).
- For accurate test results, it is essential to use clean glassware and to pipet and rinse carefully and strictly adhere to the incubation times when performing the test.

Protocol: ELISA test procedure

Important points before starting

- Please read „Important notes“ on page 8 before starting.

Things to do before starting

- Bring reagents to room temperature (18-25°C) immediately before use. In case of precipitated salt crystals in the Wash Buffer (10x), dissolve by gentle swirling and warming.
- Dilute Wash Buffer (10x) 1:10 in distilled water. For example, for one Test Plate dilute 25 ml Wash Buffer (10x) in 225 ml distilled water and mix.
- Serum/ plasma samples: Prior to sample analysis, with serum/plasma samples, dilute 1:20 in Sample Diluent (e.g., dilute 10 µl sample in 190 µl Sample Diluent) and mix well. Use plastic tubes or uncoated microtiter plates for dilution and pre-incubation. Change pipet tips for each sample.
- Controls are ready-to-use and do not require dilution for testing serum/plasma.
  Note: When testing milk samples in the overnight protocol, dilution of the controls directly in the test plate is required.

Preparation of milk samples

Prior to sample analysis, milk samples have to be defatted. Centrifuge whole milk samples for 10 min at 3000 x *g* at 10°C or store samples at 2–8°C overnight. Then remove the cream.

Take milk sample from underneath the cream layer. If necessary, use a different tip for sampling as for the penetration of the cream layer. Avoid milk cream being transferred to the microtitre plate wells, as this can cause non-specific reactions.

Dilute defatted milk 1:2 with Sample Diluent, for example, dilute 70 µl sample in 70 µl Sample Diluent and mix well. Change pipet tips for each sample.

Test procedure for serum and plasma samples

Please read „Things to do before starting“, page 9.

Procedure

1. Pre-incubate diluted samples for 15 min - 2 hours at room temperature (18-25°C) or overnight (14-22 hours) at 2-8°C.

   Close plastic tubes and cover pre-incubation plate (lid or adhesive foil).

2. Pipet 100 µl of Negative Control (in duplicate) and Positive Control (in duplicate) into appropriate wells of the Test Plate.

3. Pipet 100 µl of the pre-incubated sample into remaining wells and mix.

   Note: Record the positions of the controls and samples in a test protocol. The use of a multichannel pipet is recommended for the transfer of samples. Cover the Test Plate.

4. Incubate for 30 min at room temperature (18-25°C).

5. Remove solution from the wells by aspiration or tapping.

6. Rinse each well 3x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.

7. Pipet 100 µl ready to use Conjugate to each well and incubate for 30 min at room temperature (18-25°C).

8. Remove solution from wells by aspiration or tapping.

9. Rinse each well 3x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.

10. Pipet 100 µl TMB Substrate Solution to each well.

11. Incubate for 10 min at room temperature in the dark. Begin timing after the first well is filled.

12. Stop the reaction by adding 100 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.
13. Measure the OD in the plate reader at 450 nm within 20 min after stopping the reaction.

    Measuring at a reference wavelength (620–650 nm) is optional.

Test procedure for milk samples

- Please read „Things to do before starting“, page 9.
- Milk samples can be either tested using the short or the overnight protocol.
  Short protocol: The controls are ready to use. Please follow steps 2a and 4a.
  Overnight protocol: Dilute controls 1:2 and consider steps 2b and 4b.

Procedure

1. Pre-incubate diluted samples for 15 min – 2 hours at room temperature (18-25°C).

   Close plastic tubes and cover pre-incubation plate (lid or adhesive foil).

2. Dilute Kit Controls according to the chosen (short or overnight) test protocol:

2a**. short protocol**: Pipet 100 µl of Negative Control (in duplicate) and Positive Control (in duplicate) into appropriate wells of the Test Plate.

2b. **overnight protocol**: Pipet 50 µl of Sample Diluent into 4 Test Plate wells. Pipet 50 µl of Negative and Positive Control (in duplicate) into appropriate wells and mix well.

3. Pipet 100 µl of the pre-incubated samples into the Test Plate wells.

   Note: Record the positions of the controls and samples in a test protocol. The use of a multichannel pipet is recommended for the transfer of samples. Cover the Test Plate.

4. Incubate samples according to the chosen (short or overnight) test protocol:

4a. **short protocol**: Incubate for 30 min at room temperature (18-25°C)

4b. **overnight protocol**: Incubate overnight (14-22 h) at 2-8°C.

5. Remove solution from the wells by aspiration or tapping.

6. Rinse each well 3x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.

7. Pipet 100 µl ready to use Conjugate to each well and incubate for 30 min at room temperature (18-25°C).

8. Remove solution from wells by aspiration or tapping.

9. Rinse each well 3x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.

10. Pipet 100 µl TMB Substrate Solution to each well.

11. Incubate for 10 min at room temperature in the dark. Begin timing after the first well is filled.

12. Stop the reaction by adding 100 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.

13. Measure the OD in the plate reader at 450 nm within 20 min after stopping the reaction.

    Measuring at a reference wavelength (620–650 nm) is optional.

Data interpretation

Validation criteria

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≥ 0.6.
- The MV of the measured OD value for the Negative Control (NC) must be ≤ 0.25.

In case of invalid assays, the test should be repeated after carefully reading the instructions for use.

Calculation

Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the Positive Control is calculated according to the following equation:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Interpretation of the results

Data interpretation for serum and plasma samples

- **Samples with S/P ratio < 0.3 are negative.**
  Specific antibodies to *Mycobacterium avium* subsp. *paratuberculosis* could not be detected.
- **Samples with S/P ratio ≥ 0.3 and < 0.4 are suspect.**
  Samples with suspect results should be retested.
- **Samples with S/P ratio ≥ 0.4 are positive.**
  Specific antibodies to *Mycobacterium avium* subsp. *paratuberculosis* were detected.

Data interpretation for milk samples (short protocol)

- **Samples with S/P ratio < 0.2 are negative.**
  Specific antibodies to *Mycobacterium avium* subsp. *paratuberculosis* could not be detected.
- **Samples with S/P ratio ≥ 0.2 and < 0.3 are suspect.**
  Samples with suspect results should be retested.
- **Samples with S/P ratio ≥ 0.3 are positive.**
  Specific antibodies to *Mycobacterium avium* subsp. *paratuberculosis* were detected.

Data interpretation for milk samples (overnight protocol)

- **Samples with S/P ratio < 0.7 are negative.**
  Specific antibodies to *Mycobacterium avium* subsp. *paratuberculosis* could not be detected.
- **Samples with S/P ratio ≥ 0.7 are positive.**
  Specific antibodies to *Mycobacterium avium* subsp. *paratuberculosis* were detected.

INDICAL offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens.

Visit **www.indical.com** for more information about bactotype, cador, cattletype, flocktype, pigtype and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective INDICAL kit handbook or user manual.

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Change index

Handbook	Version	Change
HB-1615-EN-006	January 2021	Product LCM
HB-1615-EN-005	June 2018	INDICAL design

Quick guide for cattletype MAP Ab

Sample dilution:

Serum, plasma 1:20, mix well Milk 1:2

Step	Serum, Plasma	Milk
1. Pre-incubation	15 min - 2 h RT or o/N 2-8°C	15 min - 2 h RT
2. Transfer	100 µl/ well	
3. Incubation	30 min RT	30 min RT or o/N 2-8°C
4. Wash	3 x 300 µl	
5. Conjugate	100 µl/ well	
6. Incubation	30 min RT	
7. Wash	3 x 300 µl	
8. TMB	100 µl/ well	
9. Incubation	10 min RT	
10. Stop	100 µl/ well	
11. Read	450 nm	

Data interpretation

Sample	Negative	Suspect	Positive
Serum, plasma	S/P < 0.3	0.3 ≤ S/P < 0.4	S/P ≥ 0.4
Milk (short protocol)	S/P < 0.2	0.2 ≤ S/P < 0.3	S/P ≥ 0.3
Milk (overnight protocol)	S/P < 0.7		S/P ≥ 0.7

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