November 2014

cattletype® MAP Ab Handbook

5 (catalog no. 270803)

20 (catalog no. 270805)*

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

Registered in accordance with § 17c of the German Law on Animal Diseases (FLI-B 471)

REF 270803, 270805*

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* Available only on request.

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

In addition, QIAGEN provides high-quality, easy-to-use, and sensitive molecular solutions to enable veterinary pathogen detection and animal pathogen research. The QIAGEN veterinary portfolio includes a broad range of pathogen-specific PCR-assays and an extensive and growing ELISA portfolio. For more information, visit www.qiagen.com/Animal-and-Veterinary-Testing.

Contents

Kit Contents

***cattletype* MAP Ab**		
Catalog no.	**270803**	**270805***
Number of plates	**5**	**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)	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 60ml	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-Institut and registered in accordance with § 17c of the German Law on Animal Diseases (FLI-B 471) for use in Germany for veterinary diagnostic procedures. For veterinary use only.

Symbols

Contains reagents for <N> tests


Legal manufacturer

Lot number


Use by date


Temperature limitations for storage

Handbook

Catalog number

Material number


Protect from light


For cattle, sheep, and goat samples

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 online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION: The Stop Solution contains 0.5 M sulphuric acid.

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

Quality Control

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

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
- Device for delivery and aspiration of wash solution (optional)
- Microtiter plate absorbance reader
- Tubes or plates for diluting the samples
- Distilled water

General precautions

The user should always pay attention to the following:

- Do not expose the TMB Substrate Solution to intense light or to sunlight during the performance of 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 expiration date.
- Water from ion-exchange systems used for diluting the Wash Solution (10x) may interfere with the assay if not pure enough. Water quality of double distilled water or highly purified water (Milli-Q) is suitable.
- The use of clean glass devices, careful pipetting and rinsing during the test, and strict adherence to the indicated incubation times is essential for precise test results.

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.

Wash Buffer: 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: 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.

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.

- **Controls** are ready-to-use and do not require dilution.

Protocol 1: ELISA test procedure for serum and plasma samples

Please read "Things to do before starting", page 9.

Procedure

1. **Pre-incubate diluted samples for 1 – 2 h at room temperature or overnight (12–18 hours) at 2–8°C.**

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

2. **Pipet 100 µl Negative Control (in duplicate) and Positive Control (in duplicate) into appropriate wells.**
3. **Pipet 100 µl of the pre-incubated sample into remaining wells and mix.**

   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 Wash Buffer. Remove the buffer after each rinse.**
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 Wash Buffer. Remove the buffer after each rinse.**
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.

Protocol 2: ELISA test procedure for milk samples

Please read "Things to do before starting", page 9.

Procedure

1. **Pre-incubate diluted samples for 1 – 2 h at room temperature.**
   Close plastic tubes and cover pre-incubation plate (lid or adhesive foil).
2. **Pipet 50 µl of Sample Diluent into 4 Test Plate wells. Pipet 50 µl of Negative and Positive control into the pre-determined wells in duplicate and mix well.**
3. **Pipet 100 µl of the pre-incubated milk samples into the Test Plate wells.**
   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 overnight at 2–8°C.**
5. **Remove solution from the wells by aspiration or tapping.**
6. **Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.**
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 Wash Buffer. Remove the buffer after each rinse.**
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 ≥0.7
- The MV of the measured OD value for the Negative Control (NC) must be ≤0.2

In case of invalid assays the test should be repeated after a thorough review of 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:

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

Data interpretation for serum and plasma

Samples with the S/P ratio <0.4 are negative.
Specific antibodies to *Mycobacterium avium* subsp. *paratuberculosis* could not be detected.

Samples with the S/P ratio ≥0.4 are positive.
Specific antibodies to *Mycobacterium avium* subsp. *paratuberculosis* were detected.

Data interpretation for milk

Samples with the S/P ratio <0.6 are negative.
Specific antibodies to *Mycobacterium avium* subsp. *paratuberculosis* could not be detected.

Samples with the S/P ratio ≥0.6 are positive.
Specific antibodies to *Mycobacterium avium* subsp. *paratuberculosis* were detected.

Troubleshooting Guide

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
cattletype MAP Ab (5)	For 480 reactions: 5 Test Plates (strips), Wash Solution, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	270803
cattletype MAP Ab (20)*	For 1920 reactions: 20 Test Plates (solid), Wash Solution, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	270805
Related products		
cattletype BHV1 gB Ab (5)†	For 480 reactions: 5 Test Plates (strips), Wash Solution, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	270043
cattletype BHV1 gE Ab (5)†	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	270203
cattletype Milk Prep Kit (50)	Precipitation Reagent, Neutralization Buffer, Matrix, Elution Buffer, Spin Filters, Collection Tubes	271906

*Available only on request.
† Other kit sizes are available; see www.qiagen.com.

QIAGEN offer a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens. Visit www.qiagen.com/Animal-and-Veterinary-Testing for more information about the *bactotype*®, *cador*®, *cattletype*, *flocktype*®, *pigtype*®, and *virotype*®.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Quick Guide

Sample dilution:
Serum/plasma 1:20
Milk 1:2

Step	Serum/plasma	Milk
1. Pre-incubation	1–2 h at RT or overnight at 2–8°C	1–2 h at RT
2. Transfer	100 µl/well	
3. Incubation	30 min RT	Overnight at 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	Positive
Serum/plasma	S/P <0.4	S/P ≥0.4
Milk	S/P <0.6	S/P ≥0.6

Notes

Notes

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