VetMAX™ M. tuberculosis Complex Kit

Real-time PCR detection of mycobacteria responsible for bovine tuberculosis

Catalog Number MTBC

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Technology	Species	Sample matrices	Test type
Real-time PCR (DNA) • Duplex assay • Exogenous IPC	• Bovine • Badger • Wild boar • Cervid	Lymph node and surrounding tissue (excluding caseum, skin, and fat layer)	Individual

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The VetMAX™ M. tuberculosis Complex Kit enables real-time PCR detection of the Mycobacterium tuberculosis Complex, a group of genetically related mycobacteria that can cause tuberculosis in bovine and other species. The assay targets the *IS6110* insertion sequence that is present in the genomes of *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. canetti*, *M. caprae*, and *M. pinnipedii*.

The kit has been validated for use on DNA extracted from lymph node and surrounding tissue in bovine, badger, wild boar, and cervid species. A validation report is available by request from your sales representative or technical specialist. The customer is responsible for validation of the kit with tissues not included in the report.

The kit provides assays and reagents required for single-well, real-time PCR in which M. tuberculosis Complex and internal positive control (IPC) targets are amplified and detected using fluorescent hydrolysis probe chemistry. In this document, the term *TUB* is used to designate the *IS6110* target sequence indicative of M. tuberculosis Complex organisms.

The kit includes:

- 3 – Mix MTBC: Contains primers, probes, buffer, and enzyme for optimized duplex real-time PCR amplification of TUB and IPC targets.
- 4a – EPC MTBC: Quantified DNA template (see the Certificate of Analysis) containing the M. tuberculosis Complex target sequence (external positive control). It serves as a positive control for the real-time PCR components, and it is used to set validation criteria for test results.
- 5 – IPC MTBC: Added to each test and control sample at the lysis step of the DNA isolation procedure (exogenous IPC). It serves as a control for the DNA isolation procedure, and it is used to monitor for the presence of PCR inhibitors.

Contents and storage

Component	Amount	Storage
3 – Mix MTBC	2 × 500 µL	–30°C to –10°C
4a – EPC MTBC	2 × 90 µL	
5 – IPC MTBC	1 × 550 µL	

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
Real-time PCR instrument, one of the following:	
Applied Biosystems™ 7500 Real-Time PCR System • Precision Plate Holder for 7500 Real-Time PCR Systems (A24820) • Precision Plate Holder for 0.2 mL Tubes and Strips (4367033)	Contact your local sales office.
Applied Biosystems™ 7500 Fast Real-Time PCR System • Precision Plate Holder for 7500 Fast Real-Time PCR Systems (4359652) • 7500 Fast Precision Plate Holder, for 0.1 mL Tube Strips (A29252)	
QuantStudio™ 5 Real-Time PCR System	

Item	Source
Equipment	
Nuclease-free pipettors	MLS
Two ice buckets or refrigerated racks: • One for the PCR setup area where the PCR master mix is prepared • One for the area where DNA samples and controls are prepared	MLS
Tubes, plates, and other consumables	
MicroAmp™ Optical 96-Well Reaction Plate	4316813
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Optical 8-Tube Strip, 0.2 mL	4316567
MicroAmp™ Optical 8-Cap Strips	4323032
Reagents	
Nuclease-Free Water (not DEPC-Treated)	AM9939
1X TE Buffer	MLS
Other consumables	
Nuclease-free reagent tubes for preparing the reaction mixes	MLS
Aerosol-resistant pipette tips	MLS

Procedural guidelines

- For each real-time PCR run, include the following controls:
  - Positive control reactions—use 4a – EPC MTBC.
  - Extraction control reactions—use at least 1 mock-purified sample that has been prepared in the same DNA isolation procedure as the test samples.
  - No-template control (NTC) reactions—use nuclease-free water.
- Follow “Good laboratory practices for PCR and RT-PCR” on page 4 to prevent false positives and contamination of test samples with PCR products.

Requirements for input DNA

Table 1 Recommendations for DNA isolation

Step, process, or parameter	Recommendation
Amount of starting material for DNA isolation	Excise a maximum of 5 g of tissue that exhibits tuberculosis-typical lesions.
DNA isolation method	Methods that are validated for use with the VetMAX™ M. tuberculosis Complex Kit are available by request from Technical Support.
Modification to the DNA isolation method for test samples and the mock-purified sample	Add 5 µL of 5 - IPC MTBC to the lysis solution used for DNA isolation.
Preparation of the mock-purified sample, for use in extraction control PCRs	Prepare at least 1 mock-purified sample, using nuclease-free water as the starting material. Process the mock-purified sample concurrently in the same DNA isolation procedure that is used for test samples.

Before you begin

1. Thaw reagents and samples:
   a. Thaw 3 – Mix MTBC in an ice bucket or refrigerated rack.
   b. Thaw 4a – EPC MTBC, 5 – IPC MTBC, and DNA samples in a separate ice bucket or refrigerated rack.
2. Thoroughly mix the contents of each tube by vortexing, then briefly centrifuge.

Store thawed reagents and samples at 2–8°C until use.

Set up the PCR reactions

1. Dispense 10 µL of 3 – Mix MTBC to the required number of plate wells or tubes.
2. Add the indicated component for each reaction type.

Reaction type	Component	Volume per reaction
Test sample	Sample DNA	5.0 µL
Positive control	4a – EPC MTBC	5.0 µL
Extraction control	Mock-purified sample	5.0 µL
No-template control (NTC)	Nuclease-free water	5.0 µL

3. Seal each plate or tube, mix, then centrifuge briefly to bring the contents to the bottom of the plate wells or tubes.

Set up and run the real-time PCR instrument

1. Following the manufacturer's instructions, set up the real-time PCR run using the following parameters.
   - Reaction volume: 15 µL

- Passive reference: ROX™ dye (included in 3 – Mix MTBC)

  Note: ROX™ dye must be selected if the instrument is capable of detecting it. Real-time PCR instruments that do not detect ROX™ dye may be used without affecting the accuracy of the reading.

- Thermal cycler program:

  Table 2 Thermal cycler program: standard or fast mode

Stage	Repetitions	Temperature	Time
1	1	50°C	2 minutes
2	1	95°C	5 minutes
3	40	95°C	10 seconds
		60°C	30 seconds[1]

[1] If your thermal cycler cannot be programmed to 30 seconds, Stage 3 at 60°C (the elongation step) can be extended by 1 to 3 seconds.

2. Select or create dye detectors, then assign to each well or tube.

Target	Reporter	Quencher
TUB[1]	FAM™ dye	Non-fluorescent quencher (NFQ)
IPC[1]	VIC™ dye	

[1] TUB: M. tuberculosis Complex target. IPC: 5 – IPC MTBC target.

3. Run the appropriate thermal cycler program, collecting real-time amplification data during stage 3.

Guidelines for data analysis

- Follow the instrument user guide for raw data analysis.
- Set the thresholds for each target separately.
- See the Certificate of Analysis for the manufacturing batch of the kit to validate the run and interpret the results.

Validation criteria

Refer to the C_{tQC} values in the Certificate of Analysis for the manufacturing lot of the kit. The test is validated if the following criteria are met:

Reaction type	TUB target (FAM™ dye)	IPC target (VIC™ dye)	Interpretation
Positive control	$C_t = C_{tQC}$ TUB ± 3 C_t[1]	N/A[2]	PCR is validated.
Extraction control	$C_t > 40$	$C_t = C_{tQC}$ IPC ± 4 C_t[3]	DNA isolation is validated.
No-template control	$C_t > 40$	$C_t > 40$	PCR reagents are validated.

[1] See the EPC table in the Certificate of Analysis.
[2] N/A: not applicable; the IPC C_t value of the positive control reaction is not used for validation.
[3] See the IPC table in the Certificate of Analysis.

Interpretation of results

TUB target (FAM™ dye)	IPC target (VIC™ dye)	Interpretation
$C_t < 40$	Any value	M. tuberculosis Complex is detected.
$C_t > 40$	$C_t \leq C_t$ of extraction control + 4 C_t [1]	M. tuberculosis Complex is not detected.
$C_t > 40$	$C_t > C_t$ of extraction control + 4 C_t[1]	Invalid result.

[1] The C_t of the extraction control must be validated as described in "Validation criteria" on page 3.

Retest samples with invalid results

1. Dilute the DNA samples 1:10 in 1X TE buffer.
2. Repeat the real-time PCR procedure with 5 µL of the diluted DNA, then interpret the results as follows.

Result	Interpretation
The diluted DNA is positive for M. tuberculosis Complex.	The result is validated.
The diluted DNA is negative for M. tuberculosis Complex, and the IPC result is compliant.	
The diluted DNA is negative for M. tuberculosis Complex, but the IPC result is non-compliant.	The result is invalid.

3. For diluted samples with invalid results, repeat the DNA isolation procedure on a new aliquot of the original sample lysate.

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0015857 (English)

Revision	Date	Description
C.0	4 January 2022	• Quantification of 4a-EPC MTBC was added to the product description ("Product description" on page 1). • Amount of 3 – Mix MTBC and 5 – IPC MTBC were updated in the "Contents and storage" on page 1. • Reaction volume and thermal cycler program were updated in the "Set up and run the real-time PCR instrument" on page 2. • Applied Biosystems™ 7500 Real-Time PCR System and QuantStudio™ 5 Real-Time PCR System were added to the required materials not supplied table (see "Required materials not supplied" on page 1).
B.0	28 April 2017	• Clarified the amount of input tissue: maximum of 5g. • Changed the required number of mock-purified samples: at least 1. • Clarified that the C_t value of the IPC target in the positive control reaction is not used for validation.
A.0	5 October 2016	New document.