VetMAX™ M. tuberculosis Complex Kit

DNA extraction methods optimized for use with the kit (Cat. No. MTBC)

Pub. No. MAN0015859 Rev. E.0

IMPORTANT! In France, follow Appendix A, "Homogenize the samples in order to prepare for parallel cultivation".

Species	Sample matrices	Test type
• Bovine • Badger • Wild boar • Cervid	Lymph node and surrounding tissue lesions (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**.

WARNING! BIOHAZARD. Read the biological hazard safety information at this product's page at **thermofisher.com**. Follow all applicable local, state/provincial, and/or national regulations for working with biological samples.

Purpose of this guide

This guide describes automated and manual methods of DNA extraction from 2 g to 5 g of tissue. The methods have been validated and optimized for downstream use with the VetMAX™ M. tuberculosis Complex Kit (Cat. No. MTBC).

- Automated DNA extraction is performed using the KingFisher™ mL (up to 15 samples) or the KingFisher™ Flex instrument (96-well).
- Manual DNA extraction uses silica-based spin columns.

In France, homogenize samples according to Appendix A.

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.

Materials required for sample preparation and DNA extraction

Item	Source
Equipment	
(*Optional, but recommended*) Type II Biological Safety Cabinet (BSCII)	MLS
Peristaltic laboratory mill, one of the following, or equivalent: • Stomacher™ 80 Mark 2 (Seward 0080/000/EU) • MiniMix 100 mL (Interscience P CC 011230, W CC 012230)	Fisher Scientific 1428528 Fisher Scientific 36099502 (P CC), 36099503 (W CC)
Tissue homogenizer to prepare samples for ribolysis (with beads), one of the following, or equivalent: • Precellys™ 24 Homogenizer (Bertin) • FastPrep-24™ Instrument (MP Biomedical 116004500) • Mixer Mill 400 (Verder 207450001)	Bertin EQ03119.200.RD000.0 Fisher Scientific MP116004500 Fisher Scientific 08 418 241
Benchtop microcentrifuge	MLS
Laboratory mixer (Vortex or equivalent)	MLS
Adjustable precision micropipettors	MLS
Heat blocks at 56 ± 4°C and at 70 ± 4°C, or equivalent	MLS
Consumables	
Consumables required for the mill	MLS
Microcentrifuge tubes containing 0.5 mL of glass beads (0.5 mm-diameter or less), compatible with the homogenizer	MLS
Aerosol-resistant, nuclease-free pipette tips	MLS
DNase/RNase-free microcentrifuge tubes • 1.5 mL • 2.0 mL	MLS
Petri dish, for mincing tissue	MLS
Scalpels and metallic forceps	MLS
Reagents	
5 - IPC MTBC	From the VetMAX™ M. tuberculosis Complex Kit
Nuclease-Free Water	AM12450
1X TE	MLS
1X PBS	MLS

In France, homogenize samples according to Appendix A.

Additional materials required for automated DNA extraction (MagMAX™ CORE Nucleic Acid Purification Kit)

Item	Source
Instrument	
One of the following instruments: • KingFisher™ Flex Purification System • KingFisher™ mL Purification System • KingFisher™ Duo Prime Purification System	 5400630 5400050 5400110
Equipment and consumables	
Multi-channel micropipettors	MLS
Reagent reservoir	MLS
Consumables for the KingFisher™ Flex Purification System	
Deep Well Plates, one of the following: • KingFisher™ Flex Microtiter Deep-Well 96 plate, sterile • KingFisher™ Flex Microtiter Deep-Well 96 plate, v-bottom	 95040460 95040450
KingFisher™ 96 KF microplate (standard well plates)	97002540
KingFisher™ 96 tip comb for deep-well magnets (tip combs)	97002534 or 97002820
Consumables for the KingFisher™ mL Purification System	
Tubes and Tip Combs	97002141
Consumables for the KingFisher™ Duo Prime Purification System	
KingFisher™ Duo Combi pack for Microtiter 96 Deepwell plate (tip combs, plates, and elution strips for 96 samples)	97003530
KingFisher™ Duo Elution Strip (40 pieces)[1]	97003520
KingFisher™ Duo 12-tip comb for Microtiter 96 deep-well plate (50 pieces)[1]	97003500
KingFisher™ Flex Microtiter Deep-Well 96 plates[1]	95040460
Kits	
MagMAX™ CORE Nucleic Acid Purification Kit	A32700 or A32702

[1] Included in the KingFisher™ Duo Combi pack (Cat. No. 97003530).

Additional materials required for manual DNA extraction (QIAamp™ DNA Mini Kit)

Item	Source
QIAamp™ DNA Mini Kit	Qiagen 51304
Ethanol, 96–100%	MLS

In France, homogenize samples according to Appendix A.

Workflow

Prepare samples (page 4)

▼

Homogenize the samples (page 4)

▼

Bead-beat the homogenized samples for analysis and the milled control (page 5)

▼

Extract DNA using the method that is appropriate for your laboratory:

Automated method

Extract DNA using the MagMAX™ CORE Nucleic Acid Purification Kit (page 6)

Manual method

Extract DNA using the QIAamp™ DNA Mini Kit (page 8)

Procedural guidelines

- For optimal sample quality, store collected samples before DNA extraction as indicated.
  - Store samples at 5 ± 3°C for up to 24 hours.
  - Store samples below –16°C for up to 1 year.
  - Store samples at or below –70°C for longer-term storage.
- Use the 5 - IPC MTBC provided in the VetMAX™ M. tuberculosis Complex Kit. Do not use the IPC component from other kits or from other MTBC batches.
- Perform all steps at room temperature (21 ± 5°C) unless otherwise noted.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- After thawing reagents, thoroughly mix the contents of each tube by vortexing, then briefly centrifuge before use.
- To prevent false positives and contamination of test samples with PCR products, follow “Good laboratory practices for PCR and RT-PCR” on page 9.

Prepare samples

1. Remove the caseum, skin, and fat layer from each tissue sample, ensuring that 2 g to 5 g of prepared sample is collected.
2. Finely mince the sample in a petri dish using scalpels and forceps.
3. Transfer the minced sample to a peristaltic mill bag.
4. Start the homogenization.

Homogenize the samples

Note: In France, follow Appendix A, “Homogenize the samples in order to prepare for parallel cultivation”.

Include an extraction control at step 5, then process this control concurrently with the samples for analysis.

1. Add 1X PBS to the minced tissue until the tissue is just covered.
2. Homogenize the sample in a peristaltic laboratory mill (or equivalent) at 260 rpm for 3 minutes.
3. Allow the particles to settle (for 5–20 seconds maximum).
4. Transfer two 1.8-mL aliquots of the supernatant to 2-mL DNase/RNase-free microcentrifuge tubes, then store one of the aliquots below –16°C, in case further testing is needed.

In France, homogenize samples according to Appendix A.

5. Centrifuge the other aliquot at 12,000 × *g* for 2 minutes.

   Also centrifuge the aliquot of the milled control.

6. Discard the supernatant.

Bead-beat the homogenized samples for analysis and the milled control

1. Resuspend the pellet in 600 µL of 1X PBS or 1X TE.
2. Transfer the resuspended pellet to a DNase/RNase-free tube containing the appropriate glass beads.
3. Bead-beat the sample.

Option	Settings
Precellys™ 24 homogenizer	6,800 rpm; 3 × 30 seconds
FastPrep-24™ instrument	6 M/s; 45 seconds
Mixer Mill MM 400	30 Hz; 2 × 5 minutes

4. Centrifuge at 6,000 × *g* for 3 minutes.
5. Proceed to DNA extraction with 200 µL of the supernatant.
   - “Extract DNA using the MagMAX™ CORE Nucleic Acid Purification Kit (automated method)” on page 6
   - “Extract DNA using the QIAamp™ DNA Mini Kit (manual method)” on page 8

Transfer the remaining supernatant to a new tube, then store below –16°C.

In France, homogenize samples according to Appendix A.

Extract DNA using the MagMAX™ CORE Nucleic Acid Purification Kit (automated method)

Workflow

Prepare PK Solution (page 7)

▼

Treat the samples with MagMAX™ CORE Lysis Solution and PK Solution (page 7)

▼

Set up the processing plates (page 7)

▼

Prepare Binding/Bead/IPC Mix (page 8)

▼

Combine the samples with Binding/Bead/IPC Mix (page 8)

▼

Process samples on the instrument (page 8)

Procedural guidelines

- Before use, invert bottles of solutions and buffers to ensure thorough mixing.
- To prevent cross-contamination:
  - Cover the plate or tube strip during the incubation and shaking steps, to prevent spill-over.
  - Carefully pipet reagents and samples, to avoid splashing.
- To prevent nuclease contamination:
  - Wear laboratory gloves during the procedures. Gloves protect you from the reagents, and they protect the nucleic acid from nucleases that are present on skin.
  - Use nucleic acid-free pipette tips to handle the reagents, and avoid putting used tips into the reagent containers.
  - Decontaminate lab benches and pipettes before you begin.

Before first use of the kit

Download the script

The appropriate script for the MagMAX™ CORE Nucleic Acid Purification Kit must be installed on the instrument before first use.

1. On the MagMAX™ CORE Nucleic Acid Purification Kit product web page (at **thermofisher.com**, search by catalogue number), scroll to the **Product Literature** section.
2. Right-click the appropriate file to download the latest version of the MagMAX_CORE script for your instrument.
   - KingFisher™ Flex: **MagMAX_CORE_Flex_Express**
   - KingFisher™ mL: **MagMAX_CORE_mL_Express**
   - KingFisher™ Duo Prime: **MagMAX_CORE_Duo_Express**
3. See your instrument user guide or contact Technical Support for instructions for installing the script.

In France, homogenize samples according to Appendix A.

Prepare PK Solution

Prepare PK Solution immediately before use.

1. Prepare PK Solution—Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
1X PBS	200 µL
MagMAX™ CORE Proteinase K	10 µL
Total PK Solution	**210 µL**

2. Vortex to mix, then centrifuge briefly.

Treat the samples with MagMAX™ CORE Lysis Solution and PK Solution

1. Add 200 µL of MagMAX™ CORE Lysis Solution to a new tube.
2. Add 200 µL of sample supernatant, then pipet up and down to mix.
3. Add 210 µL of PK Solution, then vortex thoroughly.
4. Centrifuge briefly to collect contents at the bottom of the tube.
5. Incubate the sample without shaking. Use one of the following options:
   - Short lysis: Incubate for 30 minutes at 70 ± 4°C.
   - Long lysis: Incubate for 16–24 hours at 56 ± 4°C.
6. Centrifuge briefly to collect contents at the bottom of the tube.

Set up the processing plates

1. Add MagMAX™ CORE Wash Solutions and MagMAX™ CORE Elution Buffer to the indicated positions, according to your instrument.

Table 1 Plate setup: KingFisher™ Flex instrument

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	MagMAX™ CORE Wash Solution 1	500 µL
Wash Plate 2	3	Deep Well	MagMAX™ CORE Wash Solution 2	500 µL
Elution	4	Standard	MagMAX™ CORE Elution Buffer	90 µL
Tip Comb	5	Standard	Place a tip comb in the plate.	

[1] Position on the instrument.

Table 2 Tube strip setup: KingFisher™ mL instrument

Position ID	Tube strip position	Tube	Reagent	Volume per well
Wash 1	B	Tube strip	MagMAX™ CORE Wash Solution 1	500 µL
Wash 2	C		MagMAX™ CORE Wash Solution 2	500 µL
Elution	D		MagMAX™ CORE Elution Buffer	90 µL
Tip Comb	N/A	N/A	Slide the tip comb into the tip comb holder.	

In France, homogenize samples according to Appendix A.

Table 3 Plate setup: KingFisher™ Duo Prime instrument

Row ID	Row in the plate	Plate type	Reagent	Volume per well
Wash 1	B	Deep Well	MagMAX™ CORE Wash Solution 1	500 µL
Wash 2	C	Deep Well	MagMAX™ CORE Wash Solution 2	500 µL
Elution[1]	Separate tube strip[2]	Elution strip	MagMAX™ CORE Elution Buffer	90 µL
Tip Comb	H	Deep Well	Place a tip comb in the plate.	

[1] Ensure that the elution strip is placed in the correct direction in the elution block.
[2] Placed on the heating element

2. (*Optional*) If you are using the KingFisher™ Flex instrument, cover the prepared processing plates with sealing foil until they are loaded into the instrument.

Prepare Binding/Bead/IPC Mix

We recommend that you prepare new Binding/Bead/IPC Mix for each processing run.

1. Vortex the MagMAX™ CORE Magnetic Beads thoroughly to ensure that the beads are fully resuspended.
2. Prepare Binding/Bead/IPC Mix—Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
MagMAX™ CORE Binding Solution	400 µL
MagMAX™ CORE Magnetic Beads	20 µL
5 - IPC MTBC	5 µL
Total Binding/Bead/IPC Mix	**425 µL**

(Optional) Store Binding/Bead/IPC Mix at room temperature for up to 24 hours.

Combine the samples with Binding/Bead/IPC Mix

1. Transfer each treated sample to the required wells in the plate (position 1) or tube strip (position A).
2. Invert the tube of Binding/Bead/IPC Mix at least 10 times to resuspend the beads, then add 425 µL of Binding/Bead/IPC Mix to each sample.
3. Immediately proceed to process samples on the instrument (next section).

Process samples on the instrument

1. Select the appropriate script on the instrument.
   - KingFisher™ Flex: **MagMAX_CORE_Flex_Express**
   - KingFisher™ mL: **MagMAX_CORE_mL_Express**
   - KingFisher™ Duo Prime: **MagMAX_CORE_Duo_Express**
2. Start the run, then load the prepared plates in their positions when prompted by the instrument.

Store purified nucleic acid on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.

Extract DNA using the QIAamp™ DNA Mini Kit (manual method)

Before first use of the kit

Prepare Buffer AW1 and Buffer AW2 according to the manufacturer's instructions.

In France, homogenize samples according to Appendix A.

Treat the lysate with Proteinase K

1. For each sample, combine the following reagents as indicated.

   IMPORTANT! Add the reagents in the order indicated at the time of use; do not mix reagents in advance.

   a. Combine AL Buffer with 5 - IPC MTBC, then mix thoroughly.

Component	Volume per test sample	Volume per mock-purified sample
AL Buffer	200 µL	200 µL
5 - IPC MTBC	5 µL	5 µL

   b. Add the following reagents *in the order indicated*, then homogenize the sample.

Component	Volume per test sample	Volume per mock-purified sample
Bead-beating supernatant	200 µL	—
1X PBS or 1X TE	—	200 µL
Proteinase K	20 µL	20 µL

2. Incubate at 56±4°C for 16–24 hours.

Bind the DNA to the column

1. When the 56°C incubation is complete, centrifuge the samples briefly to avoid aerosol condensates.
2. Add 200 µL of 96–100% ethanol to each sample (total volume ~625 µL), vortex immediately for 15 seconds, then briefly centrifuge.
3. Insert a QIAamp™ Mini Spin Column into a collection tube, then transfer the entire sample to the spin column.
4. Cap the column, then centrifuge the assembly at 15,000 × *g* for 1 minute.
5. Discard the collection tube, then place the spin column on a new collection tube.

Wash and elute the DNA

1. Add 500 µL of Buffer AW1 to each spin column, then centrifuge at 15,000 × *g* for 1 minute.
2. Discard the collection tube, then place the column on a new collection tube.
3. Repeat step 1 and step 2 using 500 µL of Buffer AW2.
4. Centrifuge at 15,000 × *g* for 3 minutes to dry the membrane.
5. Place the column on a new microcentrifuge tube, then add 100 µL of Buffer AE.
6. Incubate at room temperature for 1 minute, centrifuge at 15,000 × *g* for 1 minute, then discard the column.

   The purified DNA is in the microcentrifuge tube.

Store the purified DNA:

- At 2–8°C for immediate use.
- Below –16°C for long-term storage.

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.

In France, homogenize samples according to Appendix A.

- 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.

Appendix A Homogenize the samples in order to prepare for parallel cultivation

This procedure complies with French standard NF U47-104.

Table 4 Additional materials required

Item	Source
4% H_2SO_4	MLS
6% NaOH	MLS
Bromothymol blue	MLS
Falcon™ 50-mL Conical Centrifuge Tubes	Fisher Scientific 14-432-22
(*Optional*) pH paper	MLS

Homogenize the samples and prepare for cultivation

Include an extraction control at step 10, then process this control concurrently with the samples for analysis.

1. Prepare a 4% H_2SO_4/2% bromothymol blue solution.
   a. Add 10–12 mL of 4% H_2SO_4 to a bottle.
   b. Aseptically add 2–3 drops of 2% bromothymol blue, until the solution is visibly yellow.
2. Add the 4% H_2SO_4/2% bromothymol blue solution to the minced sample, ensuring that the sample is just covered.
3. Homogenize the sample in a peristaltic laboratory mill (or equivalent) for 3 minutes.
4. Transfer the sample mixture to a 50-mL conical tube, to ensure thorough mixing and neutralization in step 6.
5. Incubate the sample for 7 minutes (total time of contact with the acid is 10 minutes).
6. Add 6% NaOH drop-wise, while vortexing the sample until the solution turns from yellow to green (pH 6.0–8.1).

   IMPORTANT! The specific pH range is important for cultivation of the mycobacteria. If the pH is too basic, mycobacteria grow too slowly. If the pH is too acidic, the contaminants and the rapidly growing mycobacteria grow at the expense of slow growing mycobacteria, including mycobacteria in the *Mycobacterium tuberculosis* complex.

   If needed, verify the pH using pH paper.
7. Allow the particles to settle (for 5–20 seconds maximum).
8. Transfer two 1.8-mL aliquots of the supernatant to 2-mL DNase/RNase-free microcentrifuge tubes, then store one of the aliquots below –16°C, for further testing, if needed.
9. Use the remainder of the supernatant to inoculate the appropriate culture media, following the method described in French standard NF U47-104.
10. Centrifuge the other 1.8-mL aliquot of supernatant at 12,000 × *g* for 2 minutes.

    Also centrifuge the aliquot of the milled control.

In France, homogenize samples according to Appendix A.

11. Discard the supernatant.

12. Add 1 mL of 1X TE, then vortex to resuspend the pellet.

13. Centrifuge at 12,000 × *g* for 2 minutes, then discard the supernatant.

14. (*Optional*) If the supernatant is cloudy, repeat the wash (step 12–step 13).

Follow the steps to “Bead-beat the homogenized samples for analysis and the milled control” on page 5.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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**.

In France, homogenize samples according to Appendix A.

Laboratoire Service International (LSI) | 6 Allée des Ecureuils – Parc Tertiaire du Bois-Dieu | 69380 Lissieu – France
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0015859 (English)

Revision	Date	Description
E.0	28 June 2022	• Instructions for the KingFisher™ Duo Prime Purification System were added to the document. • The script names for the KingFisher™ Flex, KingFisher™ mL, and KingFisher™ Duo Prime instruments were updated. • The protocol for the MagVet™ Universal Isolation Kit was removed from the document. • The following changes were made to the procedure for the MagMAX™ CORE Nucleic Acid Purification Kit: – The PK Buffer was replaced with 1X PBS (see "Prepare PK Solution" on page 7). PK Buffer was removed from the table of additional materials required. – The amount of Lysis Solution was changed from 355 µL to 200 µL, and options for a long lysis or short lysis were added (see "Treat the samples with MagMAX™ CORE Lysis Solution and PK Solution" on page 7). – The methods were updated to add 5 - IPC MTBC when preparing the Binding/Bead Mix. • Minor updates to terminology was done throughout the document.
D.0	30 October 2019	• Added instructions for nucleic acid purification with the MagMAX™ CORE Nucleic Acid Purification Kit. • Added the recommended speed for homogenizing samples using a peristaltic laboratory mill.
C.0	11 September 2017	Changed the treatment of samples during homogenization and Proteinase K addition. Centrifugation speed for DNA elution was changed from 6,000 to 15,000 × *g*.
B.0	27 April 2017	Changed the amount of supernatant that is stored after bead-beating the samples.
A.0	25 October 2016	New document.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.