Mycobacterium Avium Subspecies Paratuberculosis DNA Test Kit

VetMAX™-Gold MAP Detection Kit

Catalog Number A29809

Pub. No. 100036684 Rev. E

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 information

Name, intended use, and principle of the procedure

The Applied Biosystems™ VetMAX™-Gold MAP Detection Kit is a real-time PCR assay for rapid *in vitro* detection of the *Mycobacterium avium* subspecies *paratuberculosis* (MAP) DNA purified from bovine feces. MAP is the agent responsible for Johne's disease (chronic granulomatous enteritis of the small intestine).

The VetMAX™-Gold MAP Detection Kit enables diagnosis of Johne's disease in cattle. Johne's disease causes severe economic losses in the cattle industry worldwide due to reduced productivity, reproductive losses, and the eventual death or culling of infected animals.

The assay is a single-well/tube, real-time PCR in which MAP Control DNA and Xeno™ DNA Control targets are amplified and detected in real-time using fluorescent hydrolysis probe chemistry. The assay targets a unique sequence element in the MAP genome to provide highly sensitive and specific results. It does not target the IS900 element.

The VetMAX™-Gold MAP Detection Kit can be used to test pools of up to five samples. Pool size should be determined by the testing laboratory, based on the prevalence of MAP in the area from which the samples were collected. Pooled samples yielding a positive result should be tested individually to determine the infection status of each animal in the positive pool.

The kit includes:

- 25X MAP Primer Probe Mix—for optimized multiplex real-time PCR amplification of Xeno™ DNA Control and MAP DNA targets.
- MAP Control DNA—a plasmid containing the MAP DNA target sequence; serves as a positive control for the real-time PCR, and it is also used to set the cycle threshold (C_t) for evaluating test results.
- Xeno™ DNA Control—serves as an internal control for the DNA isolation process, and it is also used to monitor for the presence of PCR inhibitors.

Limitations

- Handle samples as recommended in Table 1 to prevent degradation of any DNA that is present.
- Prepare pooled samples from no more than five individual samples.
- Pooling samples may cause loss of detection sensitivity of infected animals with an individual C_t ≥35.
- DNA extraction methods should yield DNA free of PCR inhibitors, which can prevent amplification of target DNA.
- Follow "Good laboratory practices for PCR and RT-PCR" on page 6 to prevent false positive amplifications due to contamination of test samples with PCR products.

Kit contents and storage conditions

Reagents for 100 25-µL PCR tests are supplied.

Component	Amount	Storage
2X qPCR Master Mix	1.375 mL	–30°C to –10°C
25X MAP Primer Probe Mix	110 µL	
Xeno™ DNA Control (5000 copies/µL)	150 µL	
MAP Control DNA (3000 copies/µL)	20 µL	
Nucleic Acid Dilution Solution	4 x 500 µL	
Nuclease-free Water	1.75 mL	–30°C to +25°C

For Veterinary Use Only.

Required materials not supplied

Item	Source[1]
Applied Biosystems™ 7500 Fast Real-Time PCR System (96-well), running SDS software v1.4	Contact your local sales office.
QuantStudio™ 5 Real-Time PCR System, 96-well, 0.1-mL	Contact your local sales office.
Plates or tubes appropriate for the real-time PCR system (96-well)	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4366932, 4346906, or equivalent
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
MicroAmp™ Optical Adhesive Film	4311971, 4360954, or equivalent
MicroAmp™ Fast 8-Tube Strip, 0.1 mL	4358293, or equivalent
MicroAmp™ Optical 8-Cap Strips	4323032, or equivalent
7500 Fast Precision Plate Holder, for 0.1 mL Tube Strips	A29252 or equivalent
Nuclease-free pipettes and filtered pipette tips	MLS
Nuclease-free reagent tubes for preparing reaction mixes	MLS
1X Phosphate Buffered Saline (PBS), pH 7.4	MLS
2 ice buckets: • One for the PCR set-up area where the PCR reaction mix is prepared • One for the area where DNA may be present	MLS

[1] 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.

Input DNA requirements

Table 1 Sample handling recommendations

Step or process	Recommendation
Transportation/storage of samples	• Transport fecal samples at 2°C to 8°C or at –30°C to –10°C. • Process samples within 24 hours of receipt.
Preparation of individual fecal samples	• Use a physical method, such as bead beating, to lyse MAP cell walls and maximize nucleic acid recovery. • Store residual individual fecal supernatants at –20°C for further testing, if needed.
(Optional) Preparation of fecal pooled supernatants	• Prepare each individual fecal sample separately by adding 0.3 g feces to 1 mL PBS. • Mix by vortexing at moderate- to high-speed for 3 minutes, then centrifuge at 100 × *g* for 1 minute. • Pool up to 5 individual fecal supernatant samples by combining 100 µL of each sample. Pool samples before beating.[1] • Store residual individual fecal supernatants at –20°C for further testing, if needed.
Preparation of mock-purified samples (for use in extraction control PCRs)	Prepare duplicate mock-purified samples, using 1X PBS as the starting material. Process mock-purified samples with the same DNA isolation method that is used for test samples.
Proposed DNA isolation method	MagMAX™ Total Nucleic Acid Isolation Kit (Cat. No. AM1840), or an equivalent magnetic bead-based DNA purification method.
Required modifications to the DNA isolation method	Add 1 µL of Xeno™ DNA Control (5000 copies) per extraction to the lysis solution used for DNA purification.

[1] Pool size is determined by the testing laboratory.

Perform real-time PCR

1 Determine the quantity of reactions and thaw the reagents

a. Plan to include the following control reactions on each plate:
   - Duplicate positive control reactions (see step 1b)
   - Duplicate no-template control (NTC) reactions; use Nuclease-free Water in place of sample DNA

b. Prepare a MAP-Xeno™ DNA mix for the positive control.

Component	Volume
Nucleic Acid Dilution Solution	495 µL
MAP Control DNA	3 µL
Xeno™ DNA Control	2 µL

c. Plan the plate layout so that the wells containing NTCs are located as far away as possible from positive controls and test samples to prevent accidental cross-contamination.

d. Thaw reagents for PCR reagent mix in one ice bucket and controls and samples in a separate ice bucket, gently vortex each tube to mix the contents thoroughly, then briefly centrifuge to collect the solution at the bottom of the tube. Keep the reagents on ice.

2 Prepare the PCR reaction mix on ice

Combine the following components for the number of reactions required plus 10% overage.

Component	Volume per reaction
2X qPCR Master Mix	12.5 µL
25X MAP Primer Probe Mix	1.0 µL
Nuclease-free Water	3.5 µL
Total volume of real-time PCR reaction mix	**17.0 µL**

3 Set up the PCR reactions

a. Dispense 17 µL of PCR reaction mix to the appropriate wells of a PCR plate or PCR tubes on ice.

b. Add the appropriate component for each reaction type, according to the following table:

Reaction type	Component	Volume per reaction
Test sample	Sample DNA	8.0 µL
NTC	Nuclease-free Water	8.0 µL
Positive control	MAP-Xeno™ DNA mix (see step 1b)	8.0 µL
Extraction control	Mock-purified PBS (see Table 1)	8.0 µL

c. Seal each plate or tube, mix, then centrifuge briefly to bring the contents to the bottom of the reaction well.

4 Set up and run the real-time PCR instrument

For detailed information to set up and run the instrument, see the appropriate documentation for your instrument.

a. Following the manufacturer's instructions, set up the run using the following parameters:
   - Experiment type: Standard curve
   - Run mode: Standard
   - Reaction volume: 25 µL
   - ROX™ passive reference dye: included in the qPCR Master Mix
   - Reporter dyes and quenchers:

Target	Reporter	Quencher
MAP	FAM™ dye[1]	BHQ™-1 dye
Xeno™ DNA	CAL Fluor™ Orange 560 dye[2]	BHQ™-1 dye

[1] Absorbance maximum of 495 nm; emission maximum of 520 nm.

[2] Absorbance maximum of 538 nm; emission maximum of 559 nm. When using an Applied Biosystems™ Real-Time PCR system, calibrate the instrument with CAL Fluor™ Orange 560 dye. Otherwise, use the VIC™ dye detector.

b. Run the thermal cycler program and collect real-time amplification data during stage 2. Use the following thermal cycler settings:

Stage		Reps.	Temp.	Time
Enzyme activation/template denaturation	1	1	95°C	10 minutes
Amplification	2	40	95°C 60°C	15 seconds 60 seconds

Data analysis

Refer to your real-time PCR instrument user guide for instructions on how to analyze your data, using the following method.

Table 2 Data analysis

Method	Details
Use the **Control-Based Threshold** setting for data analysis.	1. Select **Manual C_T**. 2. Export ΔR_n values for the positive control reactions (MAP-Xeno™ DNA mix). 3. Average the ΔR_n at cycle 40 for all replicates of the positive control for each target independently. 4. Set the threshold for the MAP Control DNA target at 5% of the average maximum fluorescence value of the MAP Control DNA amplification signal in the positive control reactions. Example: If the average maximum fluorescence value for the MAP Control DNA target in the positive control is 3.0, set the MAP DNA threshold at 0.15. 5. Repeat step 4 for the Xeno™ DNA Control target, using a 5% threshold. Example: If the average maximum fluorescence value for the Xeno™ DNA Control target in the positive control reactions is 2.0, set the Xeno™ DNA threshold at 0.1.
Check the raw fluorescence data.	Verify that samples showing amplification in normalized data also show amplification in the raw fluorescence data.

Interpretation of test results

Table 3 Criteria for a valid real-time PCR run (control reactions)

Reaction type	C_t value for MAP DNA	C_t value for Xeno™ DNA Control
Positive control	28.5–33.5	27.5–33.5
NTC	40 (undetermined)[1]	40 (undetermined)[1]
Extraction control	40 (undetermined)[1]	29–35

[1] The run is invalid if the C_t value for either MAP DNA or Xeno™ Control DNA targets in the NTC is <40 or if the C_t value for MAP DNA target in the extraction control for MAP DNA is <40; see "Troubleshooting" on page 5.

Table 4 Individual samples: interpretation of test results

C_t value for MAP DNA	C_t value for Xeno™ DNA Control	Interpretation
<37	≤40, or undetermined[1]	MAP-positive sample
40 (undetermined)	29–35	MAP-negative sample
≥37	≤40, or undetermined	Suspect result[2]
40 (undetermined)	>35	Suspect result[2]

[1] High levels of MAP DNA in the sample can reduce the signal from the Xeno™ DNA Control, resulting in a higher C_t value for the Xeno™ DNA Control in MAP-positive samples.
[2] See Table 6 for retesting suspect results.

Table 5 Pooled samples: interpretation of test results

C_t value for MAP DNA	C_t value for Xeno™ DNA Control	Interpretation	Action
<37	≤40, or undetermined[1]	MAP-positive pool	Retest individual samples from the positive pool to determine which individual sample(s) caused the positive results. See Table 4 for interpretation of results from individual samples.
40 (undetermined)	29–35	MAP-negative pool	None required.
≥37	≤40, or undetermined	Suspect result	Repeat the DNA purification with the individual samples, then repeat the real-time PCR with each DNA sample. See Table 4 for interpretation of results from individual samples.
40 (undetermined)	>35	Suspect result	Repeat the DNA purification with the individual samples, then repeat the real-time PCR with each DNA sample. See Table 4 for interpretation of results from individual samples.

[1] High levels of MAP DNA in the sample can reduce the signal from the Xeno™ DNA Control, resulting in a higher C_t value for the Xeno™ DNA Control in MAP-positive samples.

Table 6 Assessment of suspect results (individual samples only)

<table>
<tr><th>Suspect result</th><th colspan="2">Action</th></tr>
<tr><td rowspan="3">The sample MAP C_t value is ≥37 and Xeno™ DNA Control C_t is ≤40 or undetermined.
or
The sample MAP C_t value is 40 (undetermined) and Xeno™ DNA Control C_t is >35.</td><td colspan="2">Analyze suspect DNA samples for the presence or absence of PCR inhibitors by calculating the Xeno™ DNA C_t shift:
Xeno™ DNA C_t shift = SS – XEC, where:
SS = C_t of Xeno™ DNA Control in the suspect sample, and
XEC = Average C_t of Xeno™ DNA in the extraction controls.</td></tr>
<tr><td>Workflow A
Xeno™ DNA Control C_t shift is ≥1.5</td><td>Workflow B
Xeno™ DNA Control C_t shift is <1.5</td></tr>
<tr><td>1. Repeat the real-time PCR with 2 µL of the suspect DNA sample (PCR inhibitors may be present in the DNA). If the MAP DNA C_t value is:
• <37 – The sample is MAP-positive. No further testing is required.
• ≥37 – Continue with this procedure.
2. Dilute the original diagnostic sample 1:4 with 1X PBS.
3. Repeat the DNA purification from triplicate aliquots of the diluted sample.
4. Repeat the real-time PCR with 8 µL of purified DNA for each aliquot.
5. Determine the number of samples with a MAP C_t value <40:
• 0 of 3: MAP-negative
• 1 of 3: Presumptive positive; confirm with secondary test method
• ≥2 of 3: MAP-positive</td><td>1. Repeat the DNA purification from triplicate aliquots of the original diagnostic sample.
2. Repeat the real-time PCR with 8 µL of the purified DNA for each aliquot.
3. Determine the number of reactions with a MAP C_t value <40:
• 0 of 3: MAP-negative
• 1 of 3: Presumptive positive; confirm with secondary test method
• ≥2 of 3: MAP-positive</td></tr>
</table>

Troubleshooting

<table>
<tr><th>Observation</th><th>Possible cause</th><th>Recommended action</th></tr>
<tr><td rowspan="4">Positive control reaction
MAP Control DNA—no signal
Xeno™ DNA Control—no signal</td><td>The MAP Control DNA and/or Xeno™ DNA Control were:
• Improperly handled, resulting in DNA degradation.
• Not added according to instructions.</td><td>Follow good PCR practices and follow the instructions. See “Good laboratory practices for PCR and RT-PCR” on page 6.</td></tr>
<tr><td>The 2X qPCR Master Mix was stored or handled improperly, resulting in a loss of activity.</td><td>Repeat the real-time PCR with fresh reagents.</td></tr>
<tr><td>The real-time PCR instrument was not properly set up.</td><td>See “Set up and run the real-time PCR instrument” on page 3.</td></tr>
<tr><td>The PCR reaction mix was prepared incorrectly.</td><td>Repeat the test with correctly prepared PCR reaction mix. See “Prepare the PCR reaction mix on ice” on page 3.</td></tr>
<tr><td>NTC or extraction control reaction
C_t value is <40</td><td>There was contamination during the DNA extraction or real-time PCR set-up.</td><td>• Repeat the purification or real-time PCR with fresh reagents and freshly decontaminated pipettes.
• Set up the real-time PCR in an area separate from areas used for nucleic acid purification and PCR product analysis.</td></tr>
<tr><td>Test samples
Xeno™ DNA Control—no or low signal
MAP DNA—high signal</td><td>The Xeno™ DNA Control primers and probe are at limiting concentrations in the real-time PCR. High levels of MAP DNA in a sample can reduce amplification of the Xeno™ DNA Control.</td><td>No or low signal from Xeno™ DNA Control is expected in a reaction that has a strong signal for MAP DNA.</td></tr>
<tr><td rowspan="2">Test samples
Xeno™ DNA Control—no signal
MAP DNA—no signal or suspect-range signal</td><td>Poor DNA recovery.</td><td>Repeat DNA purification of the original diagnostic sample.</td></tr>
<tr><td>The DNA samples contain PCR inhibitors.</td><td>See Table 6 (Workflow A).</td></tr>
</table>

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.

Documentation and support

Customer and technical support

In the United States, call 1-800-955-6288.

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
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VLN 432 / PCN 5066.00

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Revision history: Pub. No. 100036684

Revision	Date	Description
E	9 March 2020	• In Table 1, specified that samples must be used within 24 hours of receipt, and changed the fecal supernatant storage temperature to –20℃. • Replaced the "Explanation of symbols" section with web link (thermofisher.com/symbols-definition). • Updated boilerplate content and made minor style updates for consistency with other documents.
D	3 July 2018	Updated the list of compatible real-time PCR systems.
C	7 July 2017	Corrected typographical error; minor style updates.
B	8 March 2017	• In Table 3, corrected C_T value range for Xeno™ DNA Control and MAP DNA in the positive control reaction. • In Table 3 through Table 6, clarified that a C_T value of 40 is equivalent to "undetermined".
A	2 December 2016	New document.

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