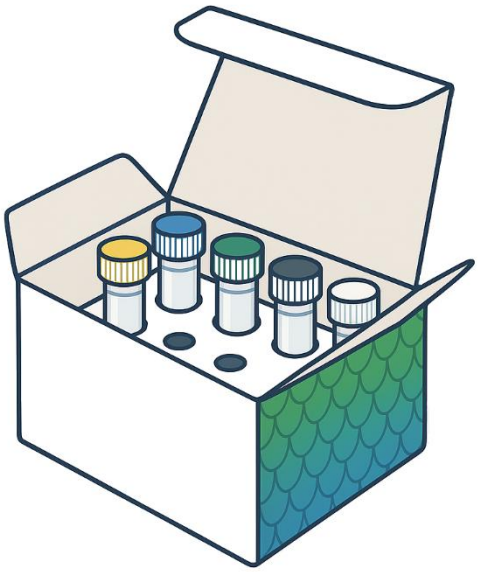
MAMBA® Diagnostik
Manufactory for Applied Molecular Biological Analytics

qPCR Listeria monocytogenes WT/139E detection Kit

Regarding ASU L 03.00-30

qPCR *Listeria monocytogenes* with 139E variant detection kit

96 tests (REF OR-002-96x) Kit version: 1



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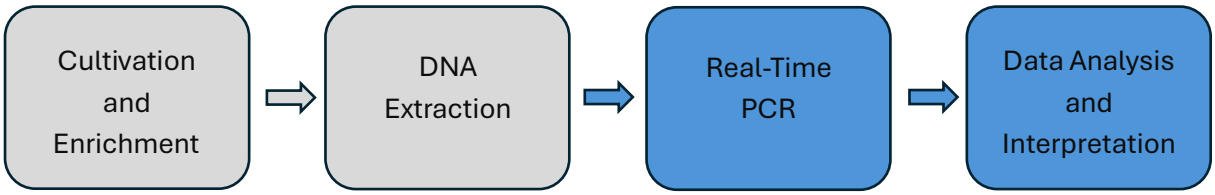
1. Product description

This qPCR *Listeria monocytogenes* with 139E variant detection kit targets the zinc metalloproteinase (mpl) gene from *Listeria monocytogenes*.
Listeria monocytogenes causes listeriosis. With Listeria contaminated food including milk and different kind of meats are the main sources of infections from Listeria. Therefore detection of this bacteria is important for animal and human health by testing food and feed products. To provide a meaningful test we provide an internal control to minimize false results. This kit detects *Listeria monocytogenes* via a specific probe which can be measured in the FAM channel after amplification. Lately a new Variant (139E) was shown to not be recognized by many kits due to differences in the mastermix. With this kit the classical *Listeria monocytogenes* as well as the variant can be reliable detected. It provides anything for the Real-Time-PCR as well as analysis and interpretation. The internal control is detected via hex channel. We advise to use the protocol DIN EN ISO 11290-1 for the cultivation and enrichment as well as the MAMBA® grey line *Listeria monocytogenes* DNA Extraction kit.
The kit is designed for 96 reactions with a final reaction volume of 20 µl including at least one positive and one negative control.



2. Specificity

The kit is designed for the in vitro quantification of *Listeria monocytogenes* genomes and to have a broad detection profile.

Due to the dynamics of genetic variation new sequence information may become available after the initial design. If you require further information or have a specific question about the detection profile of this kit, then please send an e-mail to info@mamba-diagnostik.de and our team will answer your question.
Our qPCR Kit was developed on a Applied Biosystems™ QuantStudio™ 5 from ThermoFisher and validated on a Biorad CFX Connect™ as well as an Applied Biosystems™ 7500 Fast from ThermoFisher. The kit is validated to recognize up to five copys of DNA per microliter as limit of detection (LOD).

3. Kit contents

This Kit contains following components:

	COMPONENT	COLOUR	REACTIONS INCLUDED	DETAILS
1	Mastermix	Black	96	960 µl ready-to-use ambient stable mastermix Avoid repeated freezing and thawing! Protect from light! Best storage conditions -20 °C for up to 1 year!
2	Positive control	Green	30	150 µl Positive control for *Listeria monocytogenes*
3	Positive control 2	Blue	30	150 µl positive control for the *Listeria monocytogenes* variant
4	Negative control	White	30	1 x 150 µl, contains PCR-grade water. For use as a PCR run negative control. Store at -25 to -15 °C. Optional: After first thawing, store at 2 to 8 °C for up to one month.
5	Starting solution	Yellow	96	480 µl starting solution. (Always add fresh)

4. Additional required material

- Real-time PCR instrument
- Nuclease-free, aerosol-resistant pipette filter tips
- Real-time PCR compatible plates or stripes with foil (or tubes with optical cap)
- Vortex and centrifuge for stripes or plates

5. Kit storage and stability

- Keep the kit components separate from other reagents in the laboratory.
- Use nuclease-free labware. Use fresh aerosol barrier pipette tips to avoid cross-contamination.
- Avoid pipetting into the original tube as good as possible. Transfer required solution for one experiment into a fresh tube.
- Do not prepare the assay in same place where the DNA has been extracted.
- Wear gloves when performing the assay.
- Use any sample material suitable in terms of purity and absence of inhibitors
- We recommend following the extraction protocol in MAMBA® grey line *Listeria monocytogenes* DNA Extraction kit.
- Always run a negative as well as a positive control. Use the provided controls for every test. For the negative control replace the template DNA with PCR-grade water (white colored tube). The cap of the positive control is colored in green.
- Always have an eye on our internal control. It is important to validate the test regarding the existence of inhibitors. If the internal control is not visible the test is not evaluable
- All potentially infectious or contaminated materials should be autoclaved before disposal and eliminated according the local rules and regulation. For more information refer to the appropriate safety data sheet (SDS).

! - Mastermix containing starting solution (Ready-Mastermix) is stable for one week at 4 °C.

This kit is stable at 4 °C for six months but should be stored at -20 °C (1 Year) upon arrival with maximum five thaw and freeze cycles to prolong the storage time. Unnecessary repeated freeze/thawing should be avoided.
MAMBA® Diagnostik does not recommend using the kit after the expiry date stated on the pack.

6. Enrichment and DNA extraction

This qPCR *Listeria monocytogenes* with 139E variant detection kit is developed for the detection of *Listeria monocytogenes* DNA as well as the variant V139E isolated from enrichment cultures following the DIN EN ISO 11290-1 protocol. This kit should only be used in a laboratory environment. It is not validated for diagnostic use.

7. Protocol

If the kit is stored at -20 °C thaw all the solutions, mix by flicking the tubes and spin the vials in a microcentrifuge to prevent aggregates from building up. If stored at 4 °C also flick the tubes and spin the vials in a microcentrifuge.

1. Calculate how many tests are needed
    a. Always keep positive as well as negative control in mind.
2. Pipette the appropriate amount of mastermix (10 µl per reaction) and starting solution (5 µl per Reaction) into a PCR stripe or plate.
   Mastermix with starting solution (Ready-Mastermix) is stable for one week at 4 °C.
    a. If you want to use 96 reactions you can also add the whole starting solution into the original mastermix tube.
3. Pipette 5 µl of sample per reaction. For the three controls use 5 µl PCR-graded water (NTC), 5 µl positive control 1 an in another well 5 µl positive control 2. Pipette every control into another test well.
4. Seal the tubes or plate
5. Centrifuge the tubes or plate
6. Start Real-Time-PCR with provided program

Pipetting manual per reaction

Please pipette every reaction by the following scheme:

SUBSTANCE	VOLUME
Mastermix	10 µl
Sample or positive control	5 µl
Starting solution	5 µl
total volume	20 µl

Program Setup

Program your real-time-PCR instrument to following settings:

- 95 °C 5 min (Initial denaturation)
- 95 °C 3 sec
- 60 °C 30 sec
- 72 °C 10 sec

} 45 cycles

Our test was also validated for the fast protocol on the QuantStudio™ 5 and Applied Biosystems™ 7500 Fast from ThermoFisher.

8. Analysis and interpretation

Use the following chart to interpret your results from the sample:

FAM	VIC	INTERPRETATION
+	+	Positive for *L. monocytogenes* or variant 139E
-	+	Negative for *L. monocytogenes* or variant 139E
+	-	Positive for *L. monocytogenes* negative for internal control. Take a look at the positive control. Positive control positive: Results are meaningful Positive control negative: Results are NOT meaningful
-	-	Invalid

The internal control in the positive control should always have the same value as the internal control in each test well. If the ct value deviates more than 1.5, the DNA extraction has led to the coextraction of inhibiting substances that inhibit the PCR. If in doubt, repeat the extraction and qPCR experiment.

9. Troubleshooting-guide

PROBLEM	POSSIBLE EXPLANATION	RECOMMENDATION
No signal at all, also no amplification of the internal control	PCR inhibitors are present	Repeat the PCR with different sample or extraction method. Dilute extracted DNA 1:10 to minimize inhibitor concentration.
	Incorrect detection channel has been chosen	Check the programmed method.
	Data collection was not enabled	Check the programmed method.
Negative control (NTC) shows amplification (other than internal control)	Contamination of the NTC, mastermix or starting solution with *Listeria*-DNA	Use another aliquot of every component. Repeat with a fresh batch of all reagents. Pipette positive controls after negative controls.
Varying amplification curve progression	Bubbles in PCR stripe or plate	Redo the experiment, centrifuge until all bubbles are gone. Avoid forming bubbles while pipetting.
	Signal is inhibited by fat residues on the seal or other dirt.	Always wear gloves to prevent fat residues which interfere with signal detection Also do not color the wells for easier pipetting because of intercalation with the dye from the marker.
General low fluorescence signal	Primers and probes are denatured because of inappropriate storage.	Protect the primers and probes from light. Store at -20 C for longer performance.
	Low amount of DNA or inhibitors present.	Repeat the extraction. Reconcentrate the sample

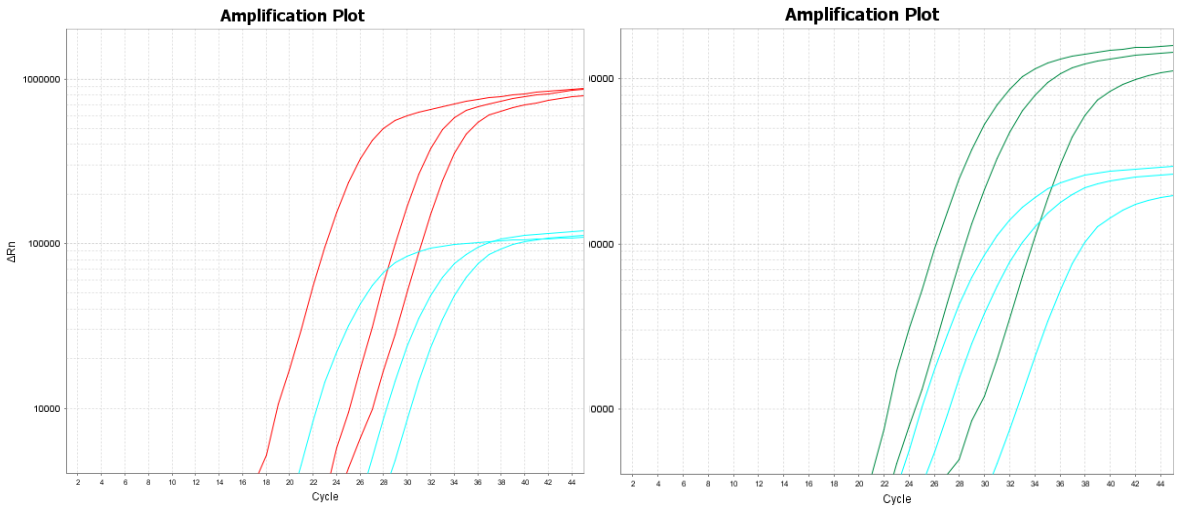


Figure 1: Amplification Plots of *Listeria monocytogenes*-DNA. Left: red= *Listeria monocytogenes*, blue= internal control. Right: green= *Listeria monocytogenes* variant 139E, blue = internal control. Both graphics show different copy amounts (1000, 100, 5 copies).

To compare your results with our validated test, please refer to Figure 1. If you have followed our instructions, your results should display comparable curves. If your test produces different results, please consult our troubleshooting guide. The shown amplification was carried out on a QuantStudio™ 5.

10. Contact

For every unclarified question or experience out of the scope of the troubleshooting-guide please contact us:

info@mamba-diagnostik.de

We will reach out to you as fast as possible with a solution. If you have other ideas to improve our product, please feel free to also contact us.