



# CERTIFICATION

## AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>

Certificate No.  
**062501**

The AOAC Research Institute hereby certifies the method known as:

**SureFast® Listeria 3plex ONE**

manufactured by

**CONGEN Biotechnologie GmbH**

**Robert Roessle Str. 10**

**D-13125 Berlin**

**Germany**

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*<sup>SM</sup> Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director  
Signature for AOAC Research Institute

Issue Date

June 11, 2025

Expiration Date

December 31, 2025

## METHOD NAME

SureFast® Listeria 3plex ONE

## CATALOG NUMBER

F5217

## ORIGINAL CERTIFICATION DATE

June 05, 2025

## PRINCIPLE OF THE METHOD

The SureFast® Listeria 3plex ONE Kit detects and differentiates *Listeria* spp. and *L. monocytogenes* in a variety of food and one environmental surface, stainless steel. The method consists of four steps: cultural enrichment, DNA-extraction, specific real-time PCR detection, and interpretation of results. A second DNA extraction kit, SureFast® PREP Bacteria Kit, can also be used with SureFast Listeria 3plex ONE Kit to provide an alternative option for DNA extraction.

The SureFast Listeria 3plex ONE Kit real-time PCR assay can be performed with commonly used real-time PCR instruments, equipped for detection of four fluorescence emissions in the channels FAM, VIC/HEX, ROX and Cy5 at the same time. Each PCR reaction contains an internal amplification control (IAC). If the DNA extract contains PCR-inhibiting substances, the signal of the amplification control can be affected, or the amplification will be suppressed. Examples for PCR-inhibiting substances are alcohols (e.g., ethanol, isopropanol), surfactants (e.g., cetrimonium bromide, SDS, Triton X-100) and salts (e.g., sodium chloride). In addition, spices, herbs, algae, cocoa and other sample matrices can have PCR-inhibiting effects.

**CERTIFIED CLAIM STATEMENT:** The SureFast® Listeria 3plex ONE method is certified for the detection of *L. grayi*, *L. fleischmannii* subsp. *fleischmannii*, *L. innocua*, *L. ivanovii*, *L. marthii*, *L. rocourtiae*, *L. seeligeri*, *L. welshimeri*, and *L. weihenstephanensis* and *L. monocytogenes* within the scope of Tables 1 and 2.

### Certified method includes:

1. Bio-Rad CFX96 Deep Well DX, Bio-Rad Opus Deepwell and R-Biopharm RIDA®CYCLER.
2. SureFast® Listeria 3plex ONE Lysis and SureFast® PREP Bacteria Lysis.

**Table 1. Method Performance Claims**

Matrix	Test Portion	Enrichment Conditions				Reference Method <sup>b</sup>	Claim <sup>c</sup>
		Broth <sup>a</sup>	Volume	Temperature	Time		
Frankfurter sausage	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Deli ham	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Smoked deli turkey	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Smoked salmon	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Frozen cooked shrimp	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Raw milk	25 mL	pw Half Fraser <sup>d</sup>	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Liquid whole egg	25 g	pw Half Fraser <sup>d</sup>	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Pasteurized milk	25 mL	pw Half Fraser <sup>d</sup>	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Vanilla ice cream	25 g	pw Half Fraser <sup>d</sup>	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Gorgonzola cheese	25 g	pw Half Fraser <sup>d</sup>	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD

Gouda cheese	25 g	pw Half Fraser <sup>d</sup>	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Brie cheese	25 g	pw Half Fraser <sup>d</sup>	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Bagged salad	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Canned Enoki mushrooms	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Ground soy vegan meat substitute	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Guacamole	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Stainless steel	1" x 1", swab <sup>e</sup>	pw Half Fraser	9 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Stainless steel	4" x 4", sponge <sup>e</sup>	pw Half Fraser	90 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Process water	25 mL	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD

<sup>a</sup> Half Fraser prewarmed.

<sup>b</sup> pw = prewarming.

<sup>c</sup> ISO = International Organization for Standardization.

<sup>d</sup> NSDD = No statistical difference detected using SLV study design from OMA Appendix J (2012). The SLV qualitative method comparison study design from OMA Appendix J (2012) is not intended to demonstrate statistical equivalence. Expert opinion is that the method is appropriate for its intended use.

<sup>e</sup> Transfer 0.1 mL Half Fraser to 10 mL Fraser broth (without ferric ammonium citrate), mix well by vortex and incubate at 37 ± 1°C for 24 ± 2 h.

<sup>f</sup> Swabs and sponges premoistened in 1 mL and 10 mL Hi-Cap Neutralizing Broth, respectively.

**Table 2. Method Selectivity**

Target	Lysis Method	Broth	Temperature	Inclusivity Strains		Exclusivity Strains	
				No. Tested	No. Positive	No. Tested	No. Positive
<i>Listeria</i> spp.	SureFast® <i>Listeria</i> 3plex ONE	Half Fraser	37 ± 1°C	106 <sup>a</sup>	100 <sup>b</sup>	30 <sup>c</sup>	0
<i>Listeria</i> spp.	SureFast® PREP Bacteria	Half Fraser	37 ± 1°C	106	101 <sup>d</sup>	30	0
<i>L. monocytogenes</i>	SureFast® <i>Listeria</i> 3plex ONE	Half Fraser	37 ± 1°C	51 <sup>e</sup>	51	30	0
<i>L. monocytogenes</i>	SureFast® PREP Bacteria	Half Fraser	37 ± 1°C	51	51	30	0

<sup>a</sup> Comprising 10 strains *L. innocua*, 6 strains *L. grayi*, 8 strains *L. ivanovii*, 9 strains *L. seeligeri*, 5 strains *L. welshimeri*, 51 strains *L. monocytogenes* and 17 additional *Listeria* spp.

<sup>b</sup> Strains not detected: *L. grandensis*, *L. floridensis*, *L. rocourtiae*, *L. cornellensis*, *L. aquatica*, and *L. portnoyi*.

<sup>c</sup> Comprising 28 species.

<sup>d</sup> Strains not detected: *L. grandensis*, *L. floridensis*, *L. cornellensis*, *L. aquatica*, and *L. portnoyi*.

<sup>e</sup> Comprising 51 strains of *Listeria monocytogenes* including 2 strains ser. 1, 7 strains ser. 1/2a, 5 strains ser. 1/2b, 3 strains ser. 1/2c, 1 strain ser. 2, 2 strains ser. 3, 1 strains ser. 3a, 2 strains ser. 3b, 1 strain ser. 3c, 2 strain ser. 4a, 10 strains ser. 4b, 2 strains ser. 4c, 1 strain ser. 4d, 1 strain ser. 4e, 1 strain ser. 7.

**Table 3. Method History**

No.	Date	Summary	Supporting Data
1	May 2025	Original Certification	Certification Report