



# CERTIFICATION

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

Certificate No.

**052602**

The AOAC Research Institute hereby certifies the method known as

**SureFast<sup>®</sup> STEC 4plex 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'.

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Bradley A. Stawick, AOAC Research Institute Senior Director

Issue Date                      June 02, 2026

Expiration Date                December 31, 2026

METHOD NAME	CATALOG NUMBER	ORIGINAL CERTIFICATION DATE
SureFast® STEC 4plex ONE	F5265, F5167, F5168	May 28, 2026

**PRINCIPLE OF THE METHOD**

The SureFast® STEC 4plex ONE Kit, SureFast® Escherichia coli Serotype I 4plex Kit, and SureFast® Escherichia coli Serotype II 4plex Kit use real-time PCR for the detection and serotyping of STEC in select foods. A second DNA extraction kit, the SureFast® PREP Bacteria Kit, can also be used with all three kits to provide an alternative option for DNA extraction. The SureFast STEC 4plex ONE detects and differentiates specific DNA sequences for the virulence factors *stx1* (subtype a-d), *stx2* (subtype a-g), *eae*, and *E. coli* O157:H7 in foods. The SureFast Escherichia coli Serotype I 4plex Kit detects and differentiates specific DNA sequences of *E. coli* serotypes O121, O26, O103. The SureFast Escherichia coli Serotype II 4plex Kit detects and differentiates specific DNA sequences of *E. coli* serotype O45, O111, and of O145.

All PCR Kits can be performed with commonly used real-time PCR instruments equipped for detection of four fluorescence emissions at the channels FAM, VIC/HEX, ROX and Cy5 at the same time. Each PCR reaction contains an internal amplification control (IAC). If the DNA contains PCR inhibiting substances, the signal of the amplification control will be affected, or the amplification will be suppressed. Examples of PCR inhibiting substances are alcohols (e.g., ethanol, isopropanol), surfactants (e.g., CTAB, SDS, Triton X100) and salts (e.g., sodium chloride).

**CERTIFIED CLAIM STATEMENT:** The SureFast® STEC 4plex ONE method is certified for the detection of Shiga-toxin producing *Escherichia coli* species and serotypes (O121, O26, O103, O45, O111, O145) within the scope of Tables 1 and 2.

**Certificated method includes:**

1. Bio-Rad CFX96 Deep Well DX, Bio-Rad Opus Deepwell and R-Biopharm RIDA®CYCLER
2. SureFast® STEC 4plex ONE Lysis, SureFast® PREP Bacteria Lysis, SureFast® E. coli Serotype I 4plex, and SureFast® E. coli Serotype II 4plex kits

**Table 1. Method performance claims**

Matrix	Test portion	Enrichment conditions				Reference method <sup>b,c</sup>	Claim <sup>d</sup>
		Broth <sup>a</sup>	Volume	Temperature	Time		
Raw beef trim	25 g	BPW	225 mL	41.5 ± 1°C	18-24 h	MLG 5C.04	NSDD
Raw beef trim	25 g	mTSB	225 mL	41.5 ± 1°C	18-24 h	MLG 5C.04	NSDD
Raw beef trim	375 g	BPW	1125 mL	41.5 ± 1°C	18-24 h	MLG 5C.04	NSDD
Raw beef trim	375 g	mTSB	1125 mL	41.5 ± 1°C	18-24 h	MLG 5C.04	NSDD
Raw ground beef (80% lean)	25 g	BPW	225 mL	41.5 ± 1°C	18-24 h	MLG 5C.04	NSDD
Raw ground beef (80% lean)	25 g	mTSB	225 mL	41.5 ± 1°C	18-24 h	MLG 5C.04	NSDD
Raw ground beef (80% lean)	375 g	BPW	1125 mL	41.5 ± 1°C	18-24 h	MLG 5C.04	NSDD
Raw ground beef (80% lean)	375 g	mTSB	1125 mL	41.5 ± 1°C	18-24 h	MLG 5C.04	NSDD
Baby spinach	200 g	BPW	1800 mL	41.5 ± 1°C	18-24 h	BAM Ch. 4A (2020)	NSDD
Sprouts	200 g	BPW	1800 mL	41.5 ± 1°C	18-24 h	BAM Ch. 4A (2020)	NSDD

Guacamole	25 g	BPW	225 mL	41.5 ± 1°C	18-24 h	BAM Ch. 4A (2020)	NSDD
Wheat flour	25 g	BPW	225 mL	41.5 ± 1°C	18-24 h	BAM Ch. 4A (2020)	NSDD

<sup>a</sup> BPW = Buffered Peptone Water (ISO formulation); mTSB = Modified Tryptic Soy Broth (ISO formulation)

<sup>b</sup> MLG = Microbiological Laboratory Guidebook

<sup>c</sup> BAM = Bacteriological Analytical Manual

<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.

**Table 2. Method selectivity**

Lysis Method	Broth <sup>a</sup>	Temperature	Inclusivity strains <sup>b</sup>		Exclusivity strains <sup>c</sup>	
			No. tested	No. positive	No. tested	No. positive
SureFast® STEC 4plex ONE	BPW	41.5 ± 1°C	100	100	30	0
SureFast® STEC 4plex ONE	mTSB	41.5 ± 1°C	100	100	30	0
SureFast® Escherichia coli Serotype I 4plex	BPW	41.5 ± 1°C	25	25	NA <sup>d</sup>	NA
SureFast® Escherichia coli Serotype I 4plex	mTSB	41.5 ± 1°C	25	25	NA	NA
SureFast® Escherichia coli Serotype II 4plex	BPW	41.5 ± 1°C	25	25	NA	NA
SureFast® Escherichia coli Serotype II 4plex	mTSB	41.5 ± 1°C	25	25	NA	NA

<sup>a</sup> BPW = Buffered Peptone Water (ISO formulation); mTSB = Modified Tryptic Soy Broth (ISO formulation)

<sup>b</sup> Comprising serogroups O157, O26, O45, O103, O111, O121, O145

<sup>c</sup> Comprising 30 non-target species

<sup>d</sup> NA = Not Applicable; the exclusivity strains were included with each set of inclusivity strains tested with each enrichment broth.

**Table 3. Method history**

No.	Date	Summary	Supporting data
1	May 28, 2026	Original Certification	Certification Report