CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

091301

The AOAC Research Institute hereby certifies the method known as:

BAX® System Real-Time PCR Assay Suite for STEC

manufactured by

Hygiena
2 Boulden Circle
New Castle, DE 19720 USA

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*℠ 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*℠ 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.

Bradley A. Stawick, AOAC Research Institute Senior Director

Issue Date	December 26, 2025
Expiration Date	December 31, 2026

METHOD NAME
BAX® System Real-Time PCR Assay Suite for STEC
Formerly DuPont™ BAX® System Real-Time PCR Assay Suite for STEC

CATALOG NUMBERS
KIT2021, KIT2008, KIT2009

ORIGINAL CERTIFICATION DATE
September 24, 2013

PRINCIPLE OF THE METHOD

The BAX System STEC Suite is a quick and reliable method for detecting Shiga toxin-producing *E. coli* (STEC) strains. Each real-time PCR assay in the STEC Suite is designed to report clear yes/no results at concentrations as low as 10^4 cfu/mL after enrichment. The screening assay detects the STEC virulence genes (stx_1, stx_2 and eae) with a simple, single-stage enrichment; two panel assays can then use the same lysate to determine if an STEC-screening positive is also positive for one of the top six STEC serogroups (*E. coli* O26, O45, O103, O111, O121, and O145).

CERTIFIED CLAIM STATEMENT: The BAX® System Real-Time PCR Assay Suite for STEC method is certified for the detection of Shiga toxin-producing *E. coli* (STEC) strains within the scope of Tables 1 and 2 and with modifications indicated in Table 3.

Certified method includes:

1. BAX® System Q7 cycler/detector, software version 5.1

Table 1. Method Performance Claims

Matrix	Test Portion	Enrichment Conditions				Reference Method/SMPR[b]	Claim[c]
		Broth[a]	Volume	Temperature	Time		
Raw beef trim	325 g	TSBg + 10 g/L caa + 8 mg/L n	975 mL	41 ± 1°C	15-24 h	MLG 5B.01	NSDD
	375 g	pw(46°C) TSBg	1500 mL	41 ± 1°C	12-24 h	MLG 5B.01	NSDD
	375 g	pw(46°C) MP	1500 mL	41 ± 1°C	12-24 h	MLG 5B.01	NSDD
Raw ground beef	25 g	pw(37°C) BPW	225 mL	37 ± 1°C	10-24 h	MLG 5B.05	NSDD
	25 g	pw(37°C) mTSB+caa	225 mL	41 ± 1°C	10-24 h	MLG 5B.05	NSDD
	375 g	pw(46°C) TSBg + 2 mg/L n	1500 mL	41 ± 1°C	12-24 h	MLG 5B.01	SDD-C
Raw ground beef plus soy	325 g	pw(35°C) mTSBg + 10 g/L caa + 8 mg/L n	975 mL	41 ± 1°C	12-24 h	MLG 5B.01	NSDD
All-purpose flour	25 g	pw(42°C) mTSB+ 2 mg/L n	225 mL	41 ± 1°C	24 h	BAM Ch. 4A	NSDD
Dried cannabis flower	10 g	pw(37-42°C) BPW	90 mL	39 ± 2°C	22-26 h	SMPR 2020.012	NSDD
Dried hemp flower	10 g	pw(37-42°C) BPW	90 mL	39 ± 2°C	22-26 h	SMPR 2020.012	NSDD
Sampling cloth from 375 g beef trim	1 cloth	pw(42°C) MP	200 mL	42 ± 1°C	8-24 h	MLG 5C.03	NSDD
	1 cloth	pw(42°C) mTSB + 10 g/L caa	200 mL	42 ± 1°C	8-24 h	MLG 5C.03	NSDD

[a] pw=prewarmed; TSB = Tryptic Soy Broth; TSBg = Tryptic Soy Broth with 2.5 g/L glucose; n = novobiocin; caa = casamino acids, MP = BAX System MP Media, mTSB = modified Tryptic Soy Broth, BPW = Buffered Peptone Water

[b] BAM=Bacteriological Analytical Manual; MLG = Microbiology Laboratory Guidebook; SMPR = Standard Method Performance Requirements

[c] 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

			Inclusivity Strains		Exclusivity Strains	
Target	Broth[a]	Temperature	No. Tested	No. Positive	No. Tested	No. Positive
STEC sreening (*stx* & *eae*)	BHI	37 ± 1°C	26[b]	26	72[c]	0
STEC Panel 1 (O26, O111, O121)	BHI	37 ± 1°C	46[d]	46	72[c]	0
STEC Panel 2 (O45, O103, O145)	BHI	37 ± 1°C	56[e]	56	72[c]	0

[a] BHI = Brain Heart Infusion

[b] Comprising serogroups O26, O45, O103, O111, O121, O145, O157:H7 and 8 additional serogroups: O4, O5, O14, O55, O63, O125, 0165, O177

[c] Comprising 37 species and 28 non-target *E. coli*.

[d] Comprising 16 strains O26, 12 strains O111, 18 strains O121

[e] Comprising 13 strains O45, 27 strains O103, 16 strains O145

Table 3. Method History

No.	Date	Summary	Supporting Data
1	September 2013	Original certification: Includes raw beef trim, raw ground beef (325 g, 375g), raw ground beef plus soy	Certification Report
2	December 2017	Level 2 Modification: Addition of raw ground beef (25 g) and all-purpose flour	Modification 1 Report
3	January 2022	Level 2 Modification: Addition of dried cannabis flower [>0.3% delta 9-tetrahydrocannabinol (THC)] and dried hemp flower (≤0.3% THC)	Modification 2 Report
4	April 2023	Level 2 Modification: Addition of sampling cloth from 375 g beef trim	Modification 3 Report