CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

071902

The AOAC Research Institute hereby certifies the method known as:

Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*)

manufactured by

Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912 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 05, 2025
Expiration Date	December 31, 2026

METHOD NAME
Neogen® Molecular Detection Assay 2 – STEC Gene Screen (*stx* and *eae*)
Formerly 3M™ Molecular Detection Assay 2 – STEC Gene Screen (*stx* and *eae*)

CATALOG NUMBER
MDA2STXEAE48

ORIGINAL CERTIFICATION DATE
July 03, 2019

PRINCIPLE OF THE METHOD

The Neogen® Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) is used with the Neogen Molecular Detection System for the rapid and specific screening of *Escherichia coli* genes *stx*1 and/or *stx*2 and *eae* in enriched food samples. The Molecular Detection Assays use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification.

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analyzed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while Negative results will be displayed after the run is completed. Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the appropriate reference method confirmation, MLG 5C.00 or BAM Chapter 4A as relevant to the matrix.

The Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae***)** is intended for use in a laboratory environment by professionals trained in laboratory techniques. Neogen has not documented the use of this product in industries other than food. For example, Neogen has not documented this product for testing pharmaceutical, cosmetics, clinical or veterinary samples. The Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) has not been evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria. The Neogen Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the Molecular Detection Instrument.

As with all test methods, the source of enrichment medium can influence the results. The Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) has been evaluated for use with the Neogen Buffered Peptone Water (ISO Formulation) (Neogen BPW ISO)- enrichment broth.

CERTIFIED CLAIM STATEMENT: The Molecular Detection Assay 2 – STEC Gene Screen (*stx* and *eae*) method is certified for the detection of *Escherichia coli* genes *stx*1 and/or *stx*2 and *eae* within the scope of Tables 1 and 2 and the modifications indicated in Table 3.

***Certified method includes**:*

1. Molecular Detection Instrument, software version 3.0.1

Table 1. Method Performance Claims

Matrix	Test Portion	Enrichment Conditions				Reference Method/SMPR[b]	Claim[c]
		Broth[a]	Volume	Temperature	Time		
Fresh raw beef trim	375 g	pw BPW ISO	1125 mL	41.5 ± 1°C	10-18 h	MLG 5C.00	NSDD
Fresh raw ground beef	25 g	pw BPW ISO	225 mL	41.5 ± 1°C	10-18 h	MLG 5C.00	NSDD
	375 g	pw BPW ISO	1125 mL	41.5 ± 1°C	10-18 h	MLG 5C.00	NSDD
Fresh raw ground pork	375 g	pw BPW ISO	1125 mL	41.5 ± 1°C	10-18 h	MLG 5C.00	NSDD

Sampling cloth from raw beef	1 cloth	pw BPW ISO	200 mL	41.5 ± 1°C	10-24 h	MLG 5C.04	NSDD
Sampling cloth from raw pork	1 cloth	pw BPW ISO	200 mL	41.5 ± 1°C	10-24 h	MLG 5C.04	NSDD
Sampling mitt from raw chicken parts	1 mitt	pw BPW ISO	200 mL	41.5 ± 1°C	10-24 h	MLG 5C.04	NSDD
Sampling mitt from raw turkey carcass	1 mitt	pw BPW ISO	200 mL	41.5 ± 1°C	10-24 h	MLG 5C.04	NSDD
Fresh poultry parts	375 g	pw BPW ISO	1125 mL	41.5 ± 1°C	10-18 h	MLG 5C.00	NSDD
Mechanically separated chicken	25 g	pw BPW ISO	225 mL	41.5 ± 1°C	10-24 h	ISO 13136:2012	NSDD
Fresh spinach	200 g	pw BPW ISO	450 mL	41.5 ± 1°C	18-24 h	BAM 4A (2018)	NSDD
Sprouts	25 g	pw BPW ISO	225 mL	41.5 ± 1°C	18-24 h	BAM 4A (2018)	NSDD
Dried cannabis flower [>0.3% (THC)	10 g	pw BPW ISO	90 mL	41.5 ± 1°C	28-32 h	SMPR 2020.012	NSDD
Dried hemp flower (≤0.3% THC)	10 g	pw BPW ISO	90 mL	41.5 ± 1°C	28-32 h	SMPR 2020.012	NSDD

[a] BPW ISO = Buffered Peptone Water ISO formulation; pw = prewarmed

[b] MLG = Microbiology Laboratory Guidebook; BAM = Bacteriological Analytical Manual; SMPR = Standard Method Performance Requirements; ISO = International Organization for Standardization

[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	
Broth[a]	Temperature	No. Tested	No. Positive	No. Tested	No. Positive
BPW ISO	41.5 ± 1°C	50[b]	50	40[c]	0

[a] BPW ISO = Buffered Peptone Water ISO formulation

[b] Comprising serogroups O26, O45, O103, O111, O121, O145, O157:H7

[c] Comprising 25 non-*E. coli* species and 5 non-target *E. coli* serogroups (10 strains)

Table 3. Method History

No.	Date	Summary	Supporting Data
1	July 2019	Original certification: Included fresh raw beef trim, fresh raw ground beef and fresh spinach	Certification Report
2	March 2020	Level 2 Modification: Addition of fresh raw ground beef (25 g), fresh raw ground pork, fresh raw poultry parts, and sprouts	Modification 1 Report
3	January 2022	Level 2 Modification: Addition of dried cannabis flower [>0.3% (THC)] and dried hemp flower (≤0.3% THC)	Modification 2 Report
4	December 2022	Level 2 Modification: Addition of mechanically separated chicken	Modification 3 Report
5	January 2024	Level 1 Modification: Rebrand method from 3M to Neogen Corporation	NA[a]
6	February 2024	Level 2 Modification: Manufacturing location change	Modification 4 Report
7	November 2025	Level 2 Modification: Addition of sampling cloth from raw beef, sampling cloth from raw pork, sampling mitt from raw chicken parts and sampling mitt from raw turkey carcass	Modification 5 Report

[a] Not applicable