CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

101703

The AOAC Research Institute hereby certifies the method known as

Molecular Detection Assay 2 – *Cronobacter*

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	May 09, 2026
Expiration Date	December 31, 2026

METHOD NAME
Neogen® Molecular Detection Assay 2 – *Cronobacter*
Formerly 3M™ Molecular Detection Assay 2 – *Cronobacter*

CATALOG NUMBER
700002257/MDA2CRO96

ORIGINAL CERTIFICATION DATE
October 24, 2017

PRINCIPLE OF THE METHOD

The Neogen® Molecular Detection Assay 2 – *Cronobacter* is used with the Neogen Molecular Detection System for the rapid and specific detection of *Cronobacter* in enriched food and food process environmental 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. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations.

CERTIFIED CLAIM STATEMENT: The Molecular Detection Assay 2 – *Cronobacter* method is certified for the detection of *Cronobacter* species within the scope of Tables 1 and 2 and the modifications indicated in Table 3.

***Certified method includes**:*

1. Molecular Detection System Instrument
2. Optional Free DNA removal step using PMAxx™ on test portions in primary enrichment (BPW ISO) or transferring to the secondary enrichment (*Cronobacter* Selective Broth)

Table 1. Method performance claims

		Enrichment conditions					
Matrix	Test Portion	Broth[a]	Volume	Temperature	Time	Reference method[b]	Claim[c]
Powdered infant formula with probiotics	10 g	BPW ISO	90 mL	37 ± 1°C	18-24 h	ISO/FDIS 22964:2016	NSDD
	300 g	pw BPW ISO + 10 mg/L vancomycin	2700 mL	37 ± 1°C	22-24 h	ISO/FDIS 22964:2016	NSDD
	400 g	pw BPW ISO + 10 mg/L vancomycin	3600 mL	37 ± 1°C	22-24 h	ISO 22964-1:2017	NSDD
Powdered infant formula without probiotics	400 g	pw BPW ISO	3600 mL	37 ± 1°C	18-24 h	ISO 22964-1:2017	NSDD
Powdered infant cereal without probiotics	10 g	BPW ISO	90 mL	37 ± 1°C	18-24 h	ISO/FDIS 22964:2016	NSDD
	300 g	pw BPW ISO	2700 mL	37 ± 1°C	22-24 h	ISO/FDIS 22964:2016	NSDD
Lactose powder	10 g	BPW ISO	90 mL	37 ± 1°C	18-24 h	ISO/FDIS 22964:2016	NSDD

Stainless steel	4”x4”, sponge[d]	BPW ISO	90 mL	37 ± 1°C	18-24 h	ISO/FDIS 22964:2016	NSDD

[a] BPW ISO = Buffered Peptone Water ISO formulation; pw = prewarmed to 37°C

[b] ISO = International Organization for Standardization; FDIS = Final Draft International Standard

[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.

[d] Sponge was pre-moistened with 10 mL Dey/Engley neutralizing broth.

Table 2. Method selectivity

		Inclusivity strains		Exclusivity strains	
Broth[a]	Temperature	No. tested	No. positive	No. tested	No. positive
BPW ISO	37 ± 1°C	50[b]	50	30[c]	0
BPW ISO + 10 mg/L vancomycin	37 ± 1°C	50[b]	50	30[c]	0

[a] BPW ISO = Buffered Peptone Water ISO formulation

[b] Comprising *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. muytjensii*, *C. dublinensis C. universalis* and *C. condimenti*

[c] Comprising 30 species

Table 3. Method history

No.	Date	Summary	Supporting data
1	October 2017	Original certification: Included powdered infant formula with probiotics (10 g and 300 g), powdered infant cereal without probiotics (10 g and 300 g), lactose powder (10 g) and stainless steel (sponge)	Certification Report
2	2018	AOAC Official Method 2018.01: Evaluation of the 3M™ Molecular Detection Assay (MDA) 2 – *Cronobacter* for the Detection of *Cronobacter* species in Select Foods and Environmental Surfaces: Collaborative Study (First Action 2018, Final Action 2021)	OMA First Action Report
3	April 2026	Level 2 Modification: Addition of 400 g test portion size for powdered infant formula with and without probiotics and evaluation of optional free DNA removal step (PMAxx or transfer to *Cronobacter* Selective Broth).	Modification 1 Report