



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.

031601

The AOAC Research Institute hereby certifies the method known as:

Easy PlateTM EC

manufactured by

Kikkoman Biochemifa Company

2-1-1, Nishi-shinbashi

Minato-ku, Tokyo 105-0003 Japan

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM 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*SM 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, AOAC Research Institute Senior Director

Issue Date

January 21, 2026

Expiration Date

December 31, 2026

METHOD NAME

Easy Plate™ EC
Formerly known as Medi-Ca EC)

CATALOG NUMBER

61975

ORIGINAL CERTIFICATION DATE

March 27, 2016

PRINCIPLE OF THE METHOD

Easy Plate EC is a ready-made dry medium for *E. coli* and coliform count made up of four components: a waterproof sheet, a dry medium containing a gelling agent and the chromogenic enzyme substrates, 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-Gluc) and 6-bromo-5-chloro-3-indolyl-β-D-galactopyranoside (Magenta-Gal), a hydrophobic resin ring surrounding the medium, and a transparent cover over the medium. A sample suspension is dispensed on the center of the medium while the cover is lifted. After that, the cover is gently dropped to evenly spread the suspension on the medium. The suspension rapidly soaks into the medium, which turns into a gel in 3 minutes. The incubation of the sheet at 35 ± 1°C for 24 ± 1 h develops navy-blue/blue-purple and pink/red-purple colonies because of the enzymatic reaction involving the substrate: the β-glucuronidase produced by bacteria catalyzes the hydrolysis of the X-Gluc to yield an insoluble blue product and the β-galactosidase produced by bacteria catalyzes the hydrolysis of the Magenta-Gal to yield an insoluble red-purple product. Navy-blue/blue-purple colonies indicate *E. coli* and pink/red-purple colonies indicate non-*E. coli* coliform. Ninety-eight percent of *E. coli* produce both β-glucuronidase and β-galactosidase and non-*E. coli* coliform only produce β-galactosidase.

CERTIFIED CLAIM STATEMENT: The Easy Plate EC method is certified for the enumeration of *E. coli* and coliform within the scope of Tables 1 and 2 and with the modifications indicated in Table 3.

Table 1. Method Performance Claims

Matrix	Test Portion	Diluent ^a	Diluent Volume	Plate Incubation		Reference Method ^b	Target	Claim ^c
				Temperature	Time			
Raw beef	50 g	BPD	450 mL	35 ± 1°C	24 ± 1 h	OMA 966.24	Coliform	Eq
							<i>E. coli</i>	Eq
Raw pork	50 g	BPD	450 mL	35 ± 1°C	24 ± 1 h	OMA 966.24	Coliform	Eq
							<i>E. coli</i>	Eq
Raw frozen pork	50 g	BPD	450 mL	35 ± 1°C	24 ± 1 h	OMA 966.24	Coliform	Eq
							<i>E. coli</i>	Eq
Raw lamb	50 g	BPD	450 mL	35 ± 1°C	24 ± 1 h	OMA 966.24	Coliform	Eq
							<i>E. coli</i>	Eq
Cooked ham	50 g	BPD	450 mL	35 ± 1°C	24 ± 1 h	OMA 966.24	Coliform	FFP
							<i>E. coli</i>	Eq
Raw salmon	50 g	BPD	450 mL	35 ± 1°C	24 ± 1 h	OMA 966.24	Coliform	Eq
							<i>E. coli</i>	Eq
Frankfurter sausage	50 g	BPD	450 mL	35 ± 1°C	24 ± 1 h	OMA 966.24	Coliform	Eq
							<i>E. coli</i>	Eq

^a BPD = Butterfield's Phosphate-Buffered Diluent.

^b OMA = Official Methods of Analysis.

^c Eq = Equivalence of candidate and reference methods demonstrated by the $\geq 90\%$ confidence interval on difference of means contained entirely within -0.5 to 0.5 log₁₀ using SLV study design from OMA Appendix J (2012) for at least 2 of the 3 levels, including the low level, tested for that matrix. If either the medium or high level does not meet the equivalence criterion, it must have an observed DOM within -0.5 to 0.5 log₁₀. FFP = Fit for Purpose. Expert opinion is that the method is appropriate for its intended use based on statistics from OMA Appendix J (2012) that were provided.

Table 2. Method Selectivity

Inclusivity Strains		Exclusivity Strains	
No. Tested	No. Positive	No. Tested	No. Positive
51 ^a	50 ^b	41 ^c	3 ^d

^a Comprising 23 species including 25 strains *E. coli*

^b *Escherichia blattae* was not detected.

^c Comprising 37 species

^d *Aeromonas hydrophila*, *Serratia marcescens* and *Serratia rubidaea* were detected.

Table 3. Method History

No.	Date	Summary	Supporting Data
1	December 2009	Original Certification	Certification Report
2	February 2020	Level 2 Modification: Manufacturing location change from Tokyo, Japan, to Kanagawa, Japan	Modification 1 Report
3	June 2021	Level 1 Modification: Rebranded kit to reflect method developer change from Dai Nippon Printing Co., Ltd. to Kikkoman Biochemifa Company and method name change from Medi-Ca SA to Easy Plate SA	NA ^a
4	March 2022	Level 2 Modification: Manufacturing location change	Modification 2 Report
5	March 2024	Level 2 Modification: Change to the outer pouch material	Modification 3 Report

^a Not applicable