

JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials

CERTIFICATE OF ANALYSIS

ERM[®] - AD624

AGAROSE-EMBEDDED DNA PLUG FOR PFGE

	DNA fragment size		
	Fragment number	Certified value ²⁾ [kb]	Uncertainty ³⁾ [kb]
<i>Listeria monocytogenes</i> Ascl-digested DNA fragments ¹⁾	1	1106	64
	2	462.5	2.4
	3	404.1	1.9
	4	392.2	2.1
	5	249.9	1.4
	6	221.5	1.4
	7	126.2	1.1
	8	109.1	1.1
	9	77.8	0.9
	10	50.2	1.7
	11	43.7	2.7

1) As defined by the PFGE procedure described in the certification report, based on the EURL *Listeria monocytogenes* protocol (Roussel *et al.* EFSA supporting publication 2014:EN-702).

2) Certified values are values that fulfil the highest standards of accuracy. The given values represent the unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory. The certified value and its uncertainty are traceable to the International System of Units (SI).

3) The uncertainty of the certified value is the expanded uncertainty with a coverage factor $k = 2.57$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is 1 plug.

Geel, August 2019

Signed: 

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Additional Material Information

DNA fragment size

<i>Listeria monocytogenes</i> Ascl-digested DNA fragments ¹⁾	Fragment number	Value ²⁾ [kb]
	12	32.0
	13	27.8
	14	23.6

1) As defined by the PFGE procedure described in the certification report, based on the EURL *Listeria monocytogenes* protocol (Roussel *et al.* EFSA supporting publication 2014:EN-702).

2) These values refer to values that were obtained in the course of the study. They are derived from triple measurements only and are stated without an uncertainty and give merely information that may be of interest for the user. The values are traceable to the International System of Units (SI).

DESCRIPTION OF THE MATERIAL

Each unit consists of one vial containing one agarose plug for PFGE with embedded undigested DNA of *Listeria monocytogenes*. The plugs were produced and certified under the responsibility of the European Commission's Joint Research Centre. *Listeria monocytogenes* H2446 strain was used as the starting material for the production of the plugs. The plugs are stored in microvials containing TRIS-EDTA buffer solution.

ANALYTICAL METHODS USED FOR CERTIFICATION

Pulsed-field gel electrophoresis

PARTICIPANTS

Austrian Agency for Health and Food Safety (AGES), National Reference Laboratory for Listeria, Graz, AT (measurements under the scope of ISO/IEC 17025 accreditation Akkreditierung Austria, No. 0179)

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Geel, BE

Sciensano, Human Bacterial Diseases, Brussels, BE (measurements under the scope of ISO/IEC 17025 accreditation BELAC No. 81-TEST)

French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Laboratory for Food Safety, Maisons Alfort, FR (measurements under the scope of ISO/IEC 17025 accreditation Cofrac, No. 1-2246)

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, IT (measurements under the scope of ISO/IEC 17025 accreditation Accredia, No. 0111)

National Institute for Public Health – National Institute for Public Hygiene (NIPH-NIH), Food Microbiology Unit, Warsaw, PL

SAFETY INFORMATION

The usual laboratory safety measures apply.

INSTRUCTIONS FOR USE AND INTENDED USE

The material is intended for quality control and/or the assessment of method performance. As with any reference material, it can be used for establishing control charts or in validation studies.

Please note that the certified values of the material were obtained using the PFGE protocol for *Listeria* as described below. Following a different PFGE protocol may give rise to a different band pattern and therefore different results.

Pre-restriction incubation

- Incubate the plug in 100 μ L 1 x concentrated restriction enzyme (RE) buffer solution in a dry bath at 37 ± 1 °C for 10 min.

Digestion of agarose plug

- Aspirate the RE buffer and add 100 μ L RE buffer containing 5 units *Ascl* enzyme, ensure the plug is completely immersed.
- Incubate the tube in a dry bath at 37 ± 1 °C for 4 h.

PFGE of digested fragments

- Pour 0.5 x TBE buffer solution (44.5 mM Tris-Borate; 1 mM EDTA, pH 8.3) into the electrophoresis chamber, switch on the peristaltic pump and set the chiller at 14 °C.
- Prepare 1 % (w/v) Seakem gold agarose (SKG) agarose in 0.5 x TBE and keep at 50 °C until use.
- Aspirate the enzyme solution, add 200 μ L of 0.5 x TBE to the tube and incubate the sample at room temperature for 5 min.
- Place the comb on the bench top and load the plug on the bottom of the comb teeth.
- Leave the plug to dry on the comb for 3 min and seal with a drop of 1 % (w/v) SKG agarose solution.
- Position the comb in the gel tray ensuring that the lower edge of the plug is correctly aligned against the frame.
- Pour the agarose solution carefully into the gel tray, and allow the gel to solidify for 30 min.
- Remove the comb and fill the wells with 1 % (w/v) SKG agarose solution and allow to solidify.

- Use the following electrophoresis settings:
 - Initial switch time: 4 s
 - Final switch time: 40 s
 - Gradient: 6 V/cm
 - Angle: 120 °
 - Migration time: 20 h for a 14 x 13 cm (15 wells) gel
21 h for a 21 x 14 cm (30 wells) gel

- Stain the gel for 30 min under agitation (150 rpm) in 400 mL ultrapure water containing 3 x concentrated GelRed.
- Photograph the gel over a UV source using a UV orange filter.
- Process the gel image with software such as GeneTools (Syngene) or BioNumerics (Applied Maths - Biomérieux) for the delimitation of lanes, and the detection of bands.

STORAGE

The material should be stored at 4 ± 3 °C.

However, the European Commission cannot be held responsible for changes that occur during storage of the material at the customer's premises, especially of opened samples.

LEGAL NOTICE

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NOTE

A detailed certification report is available at <https://crm.jrc.ec.europa.eu/>.

A paper copy is obtainable from the Joint Research Centre, Directorate F – Health, Consumers and Reference Materials on request.



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