Instructions for Use

REF - KM0058 **Primary Listeria Agar**

Dehydrated Culture Media

1. Intended Use

KM0058 Primary Listeria Agar is a selective medium for the isolation, enumeration and presumptive identification of *Listeria* species and *L. monocytogenes* from food and environmental samples.

2. Composition*

Ingredient	g/L
Enzymatic digest of animal tissue	18.0
Enzymatic digest of casein	6.0
Yeast extract	10.0
Sodium pyruvate	2.0
Glucose	2.0
Magnesium glycerophosphate	1.0
Magnesium sulphate (anhydrous)	0.5
Sodium chloride	5.0
Lithium chloride	10.0
Disodium hydrogen phosphate (anhydrous)	2.5
5-Bromo-4-chloro-3-indolyl-ß-Dglucopyranoside	0.05
Agar	12.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

KM0058 is made according to the formulation of Ottaviani and Agosti [1]. The formulation and QC testing complies with ISO 11290:2017 [2 3] and ISO 11133:2014 [4].

4. Principle

5-Bromo-4-chloro-3-indolyl-ß-D-glucopyranoside is cleaved by the ß-glucosidase enzyme which is common to all *Listeria* species. Other organisms that possess ß-glucosidase, such as enterococci and bacilli, are inhibited by the selective agents in the medium. Differentiation between *Listeria* species is obtained through the ability of some species, such as *L. monocytogenes*, to produce a lecithinase enzyme that hydrolyses lecithin resulting in an opaque white halo around the colony [1].

Enzymatic digest of animal tissue, enzymatic digest of casein and yeast extract provide the required nitrogen and other essential nutrients. Glucose is a fermentable carbohydrate and sodium chloride maintains the osmotic balance in the medium. Sodium pyruvate and magnesium glycerophosphate promote recovery of stressed organisms. Disodium hydrogen phosphate provides buffering capacity to help maintain pH. Lithium chloride is a selective agent. To enhance selectivity, Primary Listeria Selective Supplement (E&O LS0210) containing ceftazidime, amphotericin, polymyxin B and nalidixic acid, is added. Primary Listeria Diagnostic Supplement (E&O BM8050) containing solubilised lecithin allows for the detection of lecithinase producing *Listeria* spp. primarily *L. monocytogenes*.

5. Preparation Instructions

Suspend 69.0g of dried culture medium in 950ml of deionised/purified water and mix for 10 minutes.

Autoclave to sterilise at 121°C for 15 minutes then cool in a water bath to 45-50°C and aseptically add 1 vial of selective supplement (E&O LS0210) and 50ml of Primary Listeria Diagnostic Supplement (E&O BM8050).

Gently mix the final medium before aseptically dispensing the specified volume into appropriate sterile containers and allowing to cool.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Beige fine powder	Straw firm gel
pH	N/A	7.2 ± 0.2

7. Materials Provided

KM0058 is available in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

Product Code	Product Format
KM0058-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0058-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0058-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

LS0210 Primary Listeria Selective Supplement and BM8050 Primary Listeria Diagnostic Supplement

Standard microbiological laboratory materials *e.g.,* autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

9. Specimens

KM0058 Primary Listeria Agar is suitable for the testing of the following specimens:

- Food and Environmental: products for human consumption, animal feed and environmental samples

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

Based on sample type and information provided, determine whether the specimen requires pre-enrichment prior to inoculation to the prepared medium.

Inoculate plated media directly with the sample, or subculture onto plated media after incubation in enrichment broth where required.

Food and Environmental Specimens:

For solid samples, prepare a homogenised suspension using a ratio of 1:10 of sample to Half Fraser Broth (E&O BM0647) and incubate at 30 ± 1°C aerobically for 24 to 26 hours. This primary enrichment culture may be plated directly or further enriched in Fraser Broth (incubate at 37 ± 1°C aerobically for 24 hours). With homogenised and liquid specimens, medium should be inoculated with 10µl of the enriched specimen and incubated at 37 ± 1°C aerobically for 24-48 hours.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant guidelines (eg ISO 11290-1:2017).

11. Quality Control

Organism	Incubation	Result (Specificity)
L. monocytogenes (ATCC 13932)	37 ± 1°C aerobically for 24-48 hours	Growth: Blue/green colonies with halo
L. monocytogenes (NCTC 11994)	37 ± 1°C aerobically for 24-48 hours	Growth: Blue/green colonies with halo
L. monocytogenes (NCTC 7973)	37 ± 1°C aerobically for 24-48 hours	Growth: Blue/green colonies with halo
L. innocua (NCTC 11288)	37 ± 1°C aerobically for 24-48 hours	Growth: Blue/green colonies with **no** halo
E. coli (NCTC 12241)	37 ± 1°C aerobically for 24-48 hours	Inhibited
E. coli (ATCC 8739)	37 ± 1°C aerobically for 24-48 hours	Inhibited
E. faecalis (NCTC 12697)	37 ± 1°C aerobically for 24-48 hours	Inhibited
E. faecalis (NCTC 775)	37 ± 1°C aerobically for 24-48 hours	Inhibited
S. cerevisiae (NCPF 3178)	37 ± 1°C aerobically for 24-48 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify KM0058 Primary Listeria Agar performance, dehydrated culture medium samples were used to prepare Primary Listeria Agar and tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Prepared plates were inoculated with 30-150cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated at 37 ± 1°C aerobically for 24-48 hours. All samples of prepared medium showed expected recovery and morphology of the required test organisms: *Listeria monocytogenes* (ATCC 13932), *Listeria monocytogenes* (NCTC 11994), *Listeria monocytogenes* (NCTC 7973) and *Listeria innocua* (NCTC 11288), and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241), *Escherichia coli* (ATCC 8739), *Enterococcus faecalis* (NCTC 12697), *Enterococcus faecalis* (NCTC 775) and *Saccharomyces cerevisiae* (NCPF 3178). Therefore, it can be concluded that KM0058 Primary Listeria Agar, meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Medium

- Isolates presumptively identified as *Listeria* spp. and *Listeria monocytogenes* should be subjected to further testing to confirm their identity.
- Some strains of *Listeria ivanovii* may demonstrate lecithinase activity.
- Due to natural variation, some strains may grow poorly on this medium.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations. **Refer to KM0058 Material Safety Data Sheet.** Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. Ottaviani, F., Ottaviani, M. and Agosti, M. (1997) Esperienze su un agar selettivo e differenziale per Listeria monocytogenes. Industrie Alimentari, 36(361), pp.888-889.
2. International Organization for Standardization (2017) 11290-1:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method. Geneva, ISO.
3. International Organization for Standardization (2017) 11290-2:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method. Geneva: ISO.
4. International Organization for Standardization (2014) 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. Geneva: ISO.

Version History*

001 25/09/23 - New Document Created
002 13/09/24 – QC Organisms updated

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

EC REP
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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code		Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place