

Instructions for Use

Half Fraser Broth Base

Dehydrated Culture Media

REF - KM0046

1. Intended Use

KM0046 Half Fraser Broth Base is used to make Half Fraser Broth and Fraser Broth, enrichment media for the isolation of *Listeria* species from food and environmental samples.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Enzymatic digest of animal tissue	5.0
Enzymatic digest of casein	5.0
Meat extract	5.0
Yeast extract	5.0
Sodium chloride	20.0
Disodium hydrogen phosphate	9.6
Potassium dihydrogen phosphate	1.35
Aesculin	1.0
Lithium chloride	3.0
Nalidixic acid	0.01
Acriflavine	0.0125

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Listeria monocytogenes is commonly found in soil, sewage, or faeces. It is difficult to eradicate, and can cause serious food poisoning; therefore, *L. monocytogenes* is frequently tested in food processing facilities to avoid contamination which can occur at all steps of the food manufacturing chain from raw materials to place of consumption.

Developed in 1988 by Fraser ⁽¹⁾, the formulation can be made as primary enrichment Half Fraser Broth and secondary enrichment Fraser Broth according to the requirements of ISO 11290-1:2017 ⁽²⁾.

KM0046 is recommended by the International Standardization Organization ⁽²⁾ and tested in accordance with the principals of ISO 11133:2014 ⁽³⁾.

4. Principle

The antibiotics, nalidixic acid and acriflavine, are already included in the formulation so only ferric ammonium citrate (E&O LS5004) need be added to make complete Half Fraser Broth. To prepare Fraser Broth, additional nalidixic acid and acriflavine (E&O LS5003) are required.

The peptones provide required carbon, nitrogen, and other essential nutrients in the medium. Sodium chloride maintains the osmotic balance and the phosphate buffer system helps maintain pH. *Listeria* spp. hydrolyse aesculin and the associated reaction with the Fe³⁺ ions from ferric ammonium citrate gives rise to a characteristic blackening of the medium. Lithium chloride inhibits the growth of enterococci and nalidixic acid and acriflavine are further selective agents.

5. Preparation Instructions

Suspend 55.0g of dehydrated culture medium in 1 litre of deionised/purified water and allow to mix for 10 minutes.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C.

For Half-Fraser broth: aseptically add the supplement ferric ammonium citrate (E&O LS5004).

For Fraser broth: aseptically add the supplements nalidixic acid, acriflavine and ferric ammonium citrate (E&O LS5003).

Gently but thoroughly homogenize the final medium before aseptically dispensing the specified volume into appropriate sterile containers.

The resulting product may have a slight hazy appearance, but this does not impact performance.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Straw fine powder	Yellow liquid
pH	N/A	7.2 ± 0.2

7. Materials Provided

KM0046 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
KM0046-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0046-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0046-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

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

Ingredients for preparation of media supplied by E&O Laboratories e.g., the supplements E&O LS4002 Fraser Broth Selective Supplement, E&O LS5003 Half Fraser Broth Selective Supplement, and E&O LS5004 Ferric Ammonium Citrate.

9. Specimens

KM0046 Half Fraser Broth Base is suitable for testing 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

Follow local, national, or international guidelines.

Allow the media to come to room temperature.

Food/Environmental Specimens ⁽²⁾

Detection method: According to ISO 11290-1:2017 ⁽²⁾, this process requires two selective enrichment steps in order to detect stressed *Listeria* spp. and inhibit contaminating flora respectively.

- Process the food specimen as required by relevant guidelines.
- Prepare a homogenised suspension using a ratio of 1:9 of sample to KM0046 made as Half Fraser Broth and incubate at 30°C for 25 ± 1 hour.
- After incubation with Half Fraser Broth, transfer a small sample of the broth to KM0046 made as Fraser Broth and incubate at 37°C for 24 ± 2 hours.
- After incubation with both Half Fraser Broth and Fraser Broth, inoculate specimen directly onto a selective poured plate such as E&O PP0630 *Listeria* (Oxford) Agar or E&O PP7021 Colorex™ *Listeria* Agar and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate the plates at 37 ± 1°C for 24 hours (further incubation up to 48 ± 2 hours may be required if colonies are not fully distinguished at 24 hours).

After incubation, examine broths for darkening/turbidity, indicating growth; examine subcultures on agar for colonies. Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of isolated colonies from subcultures if required. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
<i>L. monocytogenes</i> (NCTC 11994) <i>L. innocua</i> (NCTC 11288) <i>E. coli</i> (NCTC 12241) <i>E. faecalis</i> (NCTC 12697)	When made as Half Fraser Broth 30 ± 1°C aerobically for 25 ± 1 hours 30 ± 1°C aerobically for 25 ± 1 hours 30 ± 1°C aerobically for 25 ± 1 hours 30 ± 1°C aerobically for 25 ± 1 hours Subcultured 10µl of the productivity broth onto E&O PP0630 <i>Listeria</i> (Oxford) Agar and incubated at 37 ± 1°C for 24 - 48 hours.	Broth: Turbid growth. Subplate: Grey colonies with dark halo Broth: Turbid growth. Subplate: Grey colonies with dark halo Inhibited 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 KM0046 Half Fraser Broth Base performance, dehydrated culture media samples were used to prepare Half Fraser Broth and tested for turbidity, as well as colony morphology and good recovery when 10µl of incubated culture in Half Fraser Broth was subcultured onto E&O PP0630 *Listeria* (Oxford) Agar. Prepared broth samples were incubated at 30 ± 1°C for 25 ± 1

hours, and PP0630 subcultures were incubated aerobically at $37 \pm 1^\circ\text{C}$ for 24-48 hours. All samples of prepared media produced showed expected recovery and morphology of the required test organisms: *Listeria monocytogenes* (NCTC 11994) and *Listeria innocua* (NCTC 11288), and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241) and *Enterococcus faecalis* (NCTC 12697). Therefore, it can be concluded that KM0046 Half Fraser Broth Base, meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Medium

- The presence of ferric ions may interfere with certain molecular identification techniques such as PCR. Culture may be prepared for such analysis by incubation for 26 ± 2 hours at $35 \pm 1^\circ\text{C}$ in Half Fraser Broth without ferric ammonium citrate.
- Darkening of the medium may not occur even in the presence of *Listeria* species.
- Due to natural variation, some strains may grow poorly in this medium.
- Adding the supplement at a lower than recommended temperature (see Section 5) may result in the appearance of precipitation in the medium.

14. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Refer to KM0046 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 do 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. Fraser, J. and Sperber, W. (1988) Rapid Detection of *Listeria* spp. in Food and Environmental Samples by Esculin Hydrolysis. *Journal of Food Protection*, 51(10), pp.762-765.
2. International Standardization Organization (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 Standardization Organization (2014) 11133:2014 Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media. Geneva: ISO.

Version History*

- 001 13/09/23 - New Document Created
- 002 07/03/24 - Preparation: Supplement should be added at 45-50°C and mixed thoroughly. The product may have a slight hazy appearance, but this does not impact on performance. Limitation: Adding the supplement at a lower cooling temperature than recommended may result in the appearance of precipitation in the medium.

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



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TABLE OF APPLICABLE SYMBOLS

IFU/KM0046 REV. 002

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