

**LISTERIA FRASER BROTH BASE
LISTERIA FRASER BROTH BASE HALF CONCENTRATION
FERRIC AMMONIUM CITRATE SUPPLEMENT**

Powdered and ready to use media and supplement for the first and second step of *Listeria* spp. enrichment and for the enumeration of *Listeria* spp. by MPN method

TYPICAL FORMULAS**Listeria Fraser Broth Base (g/l)**

Proteose Peptone	5.000
Tryptone	5.000
Beef Extract	5.000
Yeast Extract	5.000
Sodium Chloride	20.000
Disodium Hydrogen Phosphate anhydrous	9.500
Potassium Dihydrogen Phosphate	1.350
Aesculin	1.000
Lithium Chloride	3.000
Acriflavin HCl	0.025
Nalidixic Acid	0.020

Listeria Fraser Broth Base Half Concentration (g/l)

Proteose Peptone	5.0000
Tryptone	5.0000
Beef Extract	5.0000
Yeast Extract	5.0000
Sodium Chloride	20.000
Disodium Hydrogen Phosphate anhydrous	9.500
Potassium Dihydrogen Phosphate	1.3500
Aesculin	1.0000
Lithium Chloride	3.0000
Acriflavin HCl	0.0125
Nalidixic Acid	0.0100

Ferric Ammonium Citrate Supplement (Vial contents)

	500 ml of medium	5 litres of medium
Ferric Ammonium Citrate	0.25 g	2.5 g

Directions

Suspend 27.4g of Listeria Fraser Broth Base or 27.4g of Listeria Fraser Broth Base Half Concentration in 500ml of cold distilled water. Heat to boiling until complete dissolution. Autoclave at 121°C for 15 minutes. Cool to room temperature and to each medium add the contents of one vial of Ferric Ammonium Citrate Supplement (code 4240056) reconstituted with 5ml of cold distilled water. Mix well and pour into sterile tubes or flasks under aseptic conditions.
Final pH 7.2 ± 0.2

Description

Listeria Fraser Broth Base and Listeria Fraser Broth Base Half Concentration, prepared according to the formulation described by Fraser complies with the recommendation of ISO 11290-1.

The "Half Concentration" Broth contains nalidixic acid and acriflavin at halved concentrations than Listeria Fraser Broth. Both media require the addition of the ferric ammonium citrate supplement.

Fraser Broth and Fraser Broth Half Concentration are recommended by AFNOR V08-055, ISO 11290-1, for the primary and secondary enrichment of *Listeria*, in foodstuff.

Fraser Broth is recommended by USDA-FSIS for the secondary enrichment of *Listeria* in foodstuff and environmental samples.

The presence of *Listeria* in both broths leads the blackening of the culture, due to the reaction of aesculetin, produced by the aesculin hydrolysis, with iron ions.

Technique**Primary and secondary enrichment with plating into ALOA and PALCAM or Oxford plates**

1. Make a 1:9 dilution of the sample in Fraser Broth Half Concentration (eg 25 g of sample + 225 ml of liquid medium). Incubate at 30°C for 24 hours.
2. Streak 0.1 ml aliquots of Fraser Broth Half Concentration onto a plate of ALOA medium and onto a second selective plating medium of choice (e.g. PALCAM). Incubate at 37°C for 24 ± 2 hours. Reincubate negative plates for a further 24 ± 2 hours.
3. Subculture 0.1 ml Fraser Broth Half Concentration into 10 ml of Fraser Broth and incubate at 37°C for 24 hours. If no growth occurs incubate a further 24 hours.
4. Streak 0.1 ml aliquots of Fraser Broth onto a plate of ALOA medium and onto a second selective plating medium of choice (e.g. PALCAM). Incubate for 24 ± 2 hours. Reincubate negative plates for a further 24 ± 2 hours.
5. Confirm the typical colonies with biochemical tests.

Rapid technique with one enrichment step, plating into ALOA plates and confirmation with Monocytogenes ID Discs (AFNOR validation):

1. Inoculate the sample in Fraser Broth Half Concentration in the ratio 1:10 (e.g. 25g sample + 225ml of enrichment broth).
2. Incubate at 30°C for 18-24 hours.
3. Transfer a loopful of enrichment broth to the surface of ALOA plates. Examine the plates after incubation at 37°C for 24 ± 2 hours.
4. Consider as *L. monocytogenes* the green-blue colonies surrounded by an opaque halo (typical colonies) confirmed with the rapid test Monocytogenes ID Discs (cat. n° 193005) or other suitable confirmation tests.
5. Consider as *Listeria* sp. non-*monocytogenes* the green- blue colonies without the opaque halo.
6. If no typical colonies are present after 24 h of incubation or if no growth occurs, re-incubate the plates for further 18-24 hours. If no typical colonies develop, the sample can be considered *L.monocytogenes* free. If typical colonies grow in the second period of incubation confirm these colonies as described above.

Note: Techniques for the detection of *Listeria* in foods vary, depending on the material under examination and local laws. Refer to various compendia or to national regulations for the complete procedures.

User quality assurance (37°C-24hrs)

Productivity control: *L.monocytogenes* ATCC 19111: growth and blackening

Selectivity control: *S.aureus* ATCC 25923: inhibited

Storage

Dehydrated medium: 10-30°C

User prepared tubes and flasks: 7days at 2-8°C

Ready to use tubes and flasks: 2-8°C

References

- Fraser, J.A., Sperber, W.H. (1988) J. Food Protect. **51**, 10, 762-765.
- ISO 11290-1 Microbiology of food and animal feeding stuffs-Horizontal method for the detection and enumeration of *Listeria monocytogenes* Part 1 Detection method.
- Normalisation Française, AFNOR (1993) V08-55.
- Manuel Suisse des Denrées Alimentaires, Chapitre 56, E21, juillet 2000.
- Rapporto ISTISAN 96/35 Istituto Superiore di Sanità; ISSN 1123-3117.

Packaging

4015961	Listeria Fraser Broth Base	100 g (1.8 l)
4015962	Listeria Fraser Broth Base	500 g (9.1 l)
4015963	Listeria Fraser Broth Base	5 kg (91 l)
4015941	Listeria Fraser Broth Base Half Conc.	100 g (1.8 l)
4015942	Listeria Fraser Broth Base Half Conc.	500 g (9.1 l)
4015944	Listeria Fraser Broth Base Half Conc.	5 kg (91 l)

4240056	Ferric Ammonium Citrate Supplement	10 vials, each for 500 ml of medium
42185056	Ferric Ammonium Citrate Supplement	1 vial for 5 litres of medium
551596	Listeria Fraser Broth	20 x 10ml ready to use tubes
5115962	Listeria Fraser Broth	6 x 90ml ready to use flasks
5115963	Listeria Fraser Broth	6 x 225ml ready to use flasks
5115942	Listeria Fraser Broth Base Half Conc.	6 x 90ml ready to use flasks
5115943	Listeria Fraser Broth Base Half Conc.	6 x 225ml ready to use flasks