TECHNICAL DATA SHEET

FRASER BROTH

SELECTIVE SECONDARY ENRICHMENT FOR *LISTERIA*

1 INTENDED USE

Fraser broth is used for the selective secondary enrichment of *Listeria monocytogenes* and other *Listeria* in food products, according to the standard NF EN ISO 11290-1.

2 HISTORY

The medium studied by Fraser *et al.* in 1988 is a modification of the formulation of Donnelly and Baigent. The composition of the base is identical to that of UVM Broth and was modified by the addition of lithium chloride as selective agent and of ferric ammonium citrate to visualize cultures that hydrolyze esculin, by the resultant blackening of the medium.

3 PRINCIPLES

The very good recovery of *Listeria monocytogenes* is assured by the concentration differences in nalidixic acid and acriflavin between Half-Fraser and Fraser, as well as the two enrichment steps themselves. Half-Fraser Broth allows the primary enrichment step, with secondary enrichment being performed in Fraser Broth.

Polypeptone, yeast extract and meat extract furnish the nutrients required for the growth of *Listeria*.

The high sodium chloride content increases the selectivity of the medium.

Phosphates act as buffers and maintain the pH of the medium.

Esculin is hydrolyzed by *Listeria* to glucose and esculetin, the latter compound forming a black complex with ferric ions supplied by ferric citrate, added just before use, which also favors the growth of *Listeria.*

Lithium chloride inhibits the growth of most enterococci which can also hydrolyze esculin.

Nalidixic acid blocks the DNA replication of bacteria sensitive to this antibacterial agent.

The growth of secondary Gram-positive microflora is inhibited by acriflavin.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of complete media :

- Enzymatic digest of animal tissues ..... 5,0 g
- Enzymatic digest of casein..... 5,0 g
- Yeast extract ..... 5,0 g
- Meat extract..... 5,0 g
- Sodium chloride ..... 20,0 g
- Disodium phosphate, anhydrous* ..... 9,6 g
- Monopotassium phosphate ..... 1,35 g
- Esculin..... 1,0 g
- Lithium chloride ..... 3,0 g
- Nalidixic acid ..... 20 mg
- Acriflavin (chlorhydrate) ..... 25 mg
- Ferric ammonium citrate ..... 0,50 g

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

For 55 g of dehydrated base media BK133

- Enzymatic digest of animal tissues.....5,0 g
- Enzymatic digest of casein .....5,0 g
- Yeast extract .....5,0 g
- Meat extract.....5,0 g
- Sodium chloride.....20,0 g
- Disodium phosphate, anhydrous*.....9,6 g
- Monopotassium phosphate .....1,35 g
- Esculin.....1,0 g
- Lithium chloride .....3,0 g

For one vial of supplement BS031

- Nalidixic acid .....10,00 mg
- Acriflavin (chlorhydrate).....12,5 mg
- Ferric ammonium citrate.....0,25 g

For 55 g of dehydrated base media BK115

- Enzymatic digest of animal tissues.....5,0 g
- Enzymatic digest of casein .....5,0 g
- Yeast extract .....5,0 g
- Meat extract.....5,0 g
- Sodium chloride.....20,0 g
- Disodium phosphate, anhydrous*.....9,6 g
- Monopotassium phosphate .....1,35 g
- Esculin.....1,0 g
- Lithium chloride .....3,00 g
- Nalidixic acid .....20,0 mg
- Acriflavin chlorhydrate .....25 mg

For a tube of liquid supplement BS062 (10 mL)

- Ferric ammonium citrate.....0,5 g

For a vial of liquid supplement BS059 (90 mL)

- Ferric ammonium citrate.....4,5 g

* NOTE : Equates to 12 g of Disodium hydrogen phosphate dehydrate.

5 PREPARATION

Use of dehydrated base media BK115

- Dissolve 55,0 g of base media (BK115) into 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Dispense into tubes at 10 mL per tube.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.
- Add 0,1 mL of a sterile solution of ferric ammonium citrate at 5 % (BS059 or BS062) to each broth tube.

✓ **Reconstitution : 55,0 g/L**

✓ **Sterilization : 15 min at 121 °C**

Use of dehydrated base media BK133

- Dissolve 55,0 g of base media (BK133) into 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Dispense into tubes at 10 mL per tube.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.
- Reconstitute the freeze-dried selective supplement for Fraser (BS031) with 5 mL of a 1:1 ethanol / sterile distilled water solution.
- Mix well, avoiding foam formation.
- Add 0,1 mL of the reconstituted supplement (BS031) to each broth tube.

✓ **Reconstitution : 55,0 g/L**

✓ **Sterilization : 15 min at 121 °C**

6 INSTRUCTIONS FOR USE

- Into tubes prepared as above, or into ready-to-use broth tubes (BM013), transfer 0,1 mL of the primary enrichment broth.
- Mix well.
- Incubate for 24 ± 2 hours at 37 ± 1 °C.

✓ **Inoculation : 0,1 mL into 10 mL**

✓ **Incubation : 24 h at 37 °C**

7 RESULTS

Re-inoculate all tubes (black or not) onto COMPASS *Listeria* Agar (BM123) and onto a second media of choice (PALCAM or Oxford).

NOTE
Blackening of the cultures indicates a presumptive presence of *Listeria*.
However, certain bacterial strains hydrolyze esculin, (notably enterococci), and can lead to blackening of the media.

8 QUALITY CONTROL

Dehydrated media : yellowish powder, free-flowing and homogeneous.
Prepared (complete) media : yellow-amber solutions with bluish reflections.

Typical culture response after 48 hours of incubation at 37 °C, then re-inoculation onto COMPASS *Listeria* Agar :

Microorganisms	Growth
Listeria monocytogenes 4b WDCM 00021 + *Enterococcus faecalis* WDCM 00087 + *Escherichia coli* WDCM 00013	> 10 characteristic colonies
Listeria monocytogenes ½ a WDCM 00109 + *Enterococcus faecalis* WDCM 00087 + *Escherichia coli* WDCM 00013	> 10 characteristic colonies
Enterococcus faecalis WDCM 00087 *Escherichia coli* WDCM 00013	< 100 colonies Inhibited, score 0

9 STORAGE / SHELF LIFE

Dehydrated base media (BK115) : 2-30 °C.
Dehydrated base media (BK133) : 2-30 °C.
Sterile 5% solution of ferric ammonium citrate : 2-25 °C.
Selective supplement for Fraser broth : 2-8 °C.
Ready-to-use media in tubes : 2-8 °C, shielded from light.
The expiration dates are indicated on the labels.

Prepared base media BK115 (*) : 180 days at 2-8 °C, shielded from light.
Prepared base media BK133 (*) : 180 days at 2-8 °C.
Complete prepared media in tubes (*) : 1 month at 2-8 °C, shielded from light.
Rehydrated selective supplement BS031 (*) : 30 days at 2-8 °C, shielded from light.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated FRASER broth base (without ferric ammonium citrate) :
500 g bottle BK115HA

Sterile solution of 5% ferric ammonium citrate :
10 x 90 mL vials BS05908
7 x 10 mL tubes BS06208

Dehydrated FRASER broth base II (without ferric ammonium citrate nor nalidixic acid nor acriflavin) :
500 g bottle BK133HA
5 kg drum BK133GC

Freeze dried selective supplement (with ferric ammonium citrate, nalidixic acid and acriflavin) :
10 vials qsp 500 mL BS03108

Ready-to-use media in tubes :
50 x 10 mL tubes BM01308

11 BIBLIOGRAPHY

Donnelly, C.W., and Baigent, G.J.. 1986. Method for flow cytometric detection of *Listeria monocytogenes* in milk. Applied and Environmental Microbiology, **52** : 689-695.

Fraser, J.A., and Sperber, W.H.. 1988. Rapid detection of *Listeria* spp. in food and environmental samples by esculin hydrolysis. Journal of Food Protection, **51** : 762-765.

NF EN ISO 11133. July 2014. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media (Print 2 (2016-01-01)).

NF EN ISO 11290-1. July 2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1 : detection method

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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