Technical Data Sheet

EcoCult® Half Fraser (Demi Fraser) broth (base) with antibiotics acc. ISO 11290

Ordering number: 1.40197.5000 / 1.40197.9010

For the primary selective enrichment of *Listeria spp.* including *L. monocytogenes* from food and animal feed as well as from environmental samples in the area of food production and food handling and other materials.

Half Fraser broth (Demi Fraser) broth (base) with antibiotics acc. ISO 11290 is also known as Demi Fraser broth.

This culture medium complies with the specifications given by EN ISO 11290-1 and APHA.

Mode of Action

The high nutrient content and the large buffer capacity creates optimum growth conditions for *Listeria*. The growth of accompanying bacteria is largely inhibited by lithium chloride, nalidixic acid and acriflavine hydrochloride. The detection of the ß-D-glucosidase activity is possible by the addition of aesculin and ammonium iron(III) citrate forming a black complex of aesculin with iron(III) ions. A black coloration can develop during incubation, but this reaction is not exclusive to *Listeria* spp. and cultures which show no blackening cannot be assumed to be free of *Listeria* spp. Following EN ISO 11290-1, every primary and secondary enrichment in Fraser broth has to be sub-cultured on selective plating media.

EcoCult® Half FRASER (Demi FRASER) Broth (Base) with antibiotics acc. ISO 11290 already contains the antibiotics, so it is only necessary to add the Ammonium iron(III) citrate for the preparation of Half Fraser broth.

The primary selective enrichment Half Fraser broth can be prepared by addition of one vial Ammonium iron(III) citrate supplement (article number 1.00092.0010) per 500 ml Half Fraser Broth (base).

For the secondary enrichment step, Fraser broth can be prepared by addition of one additional vial reconstituted FRASER Listeria selective supplements (article number 1.00093.0010) per 500 ml prepared Half Fraser Broth.

Typical Composition

Specified by ISO 11290		EcoCult® Half FRASER (Demi FRASER) Broth (Base) with antibiotics acc. ISO 11290	
Enzymatic Digest of Animal Tissues	5 g/l	Enzymatic Digest of Animal Tissues	5 g/l
Enzymatic Digest of Casein	5 g/l	Enzymatic Digest of Casein	5 g/l
Meat Extract	5 g/l	Meat Extract	5 g/l
Yeast Extract	5 g/l	Yeast Extract	5 g/l
NaCl	20 g/l	NaCl	20 g/l
Na_2HPO_4 x 2 H_2O	12 g/l	Na_2HPO_4, anhydrous*	9.6 g/l
KH_2PO_4	1.35 g/l	KH_2PO_4	1.35 g/l
Aesculin	1 g/l	Aesculin	1 g/l
LiCl	3 g/l	LiCl	3 g/l
Acriflavine Hydrochloride	0.0125 g/l	Acriflavine Hydrochloride	0.0125 g/l
Nalidixic Acid Sodium Salt	0.01 g/l	Nalidixic Acid Sodium Salt	0.01 g/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	7.2 ± 0.2	pH at 25 °C	7.2 ± 0.2
Supplement added after autoclaving:			
Ammonium Iron (III) Citrate	0.5 g/l	Ammonium Iron (III) Citrate	0.5 g/l

* equivalent to 12.0 g/l Na_2HPO_4 x 2 H_2O.

Preparation

Dissolve 27,5 g in 500 ml purified water and autoclave 15 min at 121 °C. Dissolve the contents of 1 vial Ammonium iron(III) citrate (article number 1.00092.0010) in 1 ml of sterile distilled water. Add to the broth after it has cooled below 50 °C.

The supplement is homogeneously distributed in the broth by carefully swirling.

For the secondary enrichment step, Fraser broth is made by adding one additional vial reconstituted FRASER Listeria selective supplements (article number 1.00093.0010) per 500 ml prepared Half Fraser Broth.

The dehydrated medium is a powder with beige colour.

The prepared medium is clear to slightly opalescent solution, may be with slightly precipitate formation. The pH value at 25 °C is in the range of 7.0 – 7.4.

Experimental Procedure and Evaluation

Depends on the purpose for which the medium is used.

For preparation of the initial suspension according to EN ISO 11290-1, use as dilution fluid the selective primary enrichment medium Half Fraser broth.

In general, to prepare the initial suspension, add a test portion of 25 g or 25 ml to 225 g or 225 ml of Half Fraser broth, to obtain a tenfold dilution, and homogenize. Pre-warm the selective primary enrichment medium to room temperature before use.

For large quantities, it is recommended to pre-warm the Half Fraser broth to (30 ± 1 °C) before mixing it with the test portion.

Incubate the inoculated Half-Fraser broth under aerobic conditions, e.g. acc. to EN ISO 11290-1 at (30 ± 1 °C) for (25 ± 1 h).

A black coloration can develop during the incubation.

After incubation of the initial suspension (primary enrichment) Half Fraser broth for (25 ± 1 h), transfer 0,1 ml of the culture obtained (regardless of its color) to a tube or bottle containing 10 ml of the selective secondary enrichment medium (Fraser broth).

Incubate for the secondary enrichment step the inoculated Fraser broth under aerobic conditions, e.g. acc. to EN ISO 11290-1 for (24 ± 2 h) at (37 ± 1 °C).

Acc. to EN ISO 11290-1, in the case of *Listeria* spp. (other than *L. monocytogenes*) detection, additional 24 h incubation can allow for recovery of more species.

Acc. to EN ISO 11290-1, Half-Fraser broth and Fraser broth can be refrigerated at (5 ± 3 °C) before isolation on selective agar for a maximum of 72 h.

From the culture obtained in the primary and the secondary selective enrichment culture selective solid media are inoculated, see details given by EN ISO 11290-1.

Storage

Store at +10 °C to +30 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

Quality Control

Function	Control strains	Incubation	Method of control	Criteria	Expected results
Productivity	*Listeria monocytogenes* ATCC® 13932 [WDCM 00021] + *Escherichia coli* ATCC® 8739 [WDCM 00012] + *Enterococcus faecalis* ATCC® 19433 [WDCM 00009]	(25 ± 1 h) at (30 ± 1 °C)	Qualitative	>10 colonies on Listeria agar acc. OTTAVIANI and AGOSTI acc. ISO 11290	Blue-green colonies with opaque halo on Listeria agar acc. OTTAVIANI and AGOSTI acc. ISO 11290
	Listeria monocytogenes ATCC® 35152 [WDCM 00109] + *Escherichia coli* ATCC® 25922 [WDCM 00013] + *Enterococcus faecalis* ATCC® 29212 [WDCM 00087]				
Selectivity	*Escherichia coli* ATCC® 8739 [WDCM 00012]	(25 ± 1 h) at (30 ± 1 °C)	Qualitative	Total inhibition on Tryptic Soy Agar (TSA)	-
	Escherichia coli ATCC® 25922 [WDCM 00013]				
	Enterococcus faecalis ATCC® 19433 [WDCM 00009]			<100 colonies on Tryptic Soy Agar (TSA)	-
	Enterococcus faecalis ATCC® 29212 [WDCM 00087]				

Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with EN ISO 11133:2014 and EN ISO 11290-1:2017.

Literature

APHA (2015): Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C.

ISO International Standardisation Organisation. Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and other *Listeria* spp. - Part 1: Detection method. EN ISO 11290:2017.

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014.

Augustin J.-C., Kalmokoff M., Ells T., Favret S., Desreumaux J., Decourseulles Brasseur E. and Gnanou Besse N. (2016): Modeling the behavior *of Listeria monocytogenes* during enrichment in half Fraser broth - Impact of pooling and the duration of enrichment on the detection of *L. monocytogenes* in food. Food Microbiol., **60**: 131–136.

Beumer, R.R. and Curtis, G.D.W. (2012): Culture media and Methods for the isolation of *Listeria monocytogenes*. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 115-129. Royal Society of Chemistry, Cambridge, UK.

Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. (2012): Handbook of Culture Media for Food and Water Microbiology, pp. 762-764. Royal Society of Chemistry, Cambridge, UK.

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

Gnanou Besse N., Favret S., Desreumaux J., Decourseulles Brasseur E. and Kalmokoff M. (2016): Evaluation of reduction of Fraser incubation by 24h in the EN ISO 11290-1 standard on detection and diversity of *Listeria* species. Int. J. Food Microbiol. **224**: 16–21.

Ordering Information

Product	Cat. No.	Pack size
EcoCult® Half Fraser (Demi Fraser) broth (base) with antibiotics acc. ISO 11290	1.40197.5000	5 kg
EcoCult® Half Fraser (Demi Fraser) broth (base) with antibiotics acc. ISO 11290	1.40197.9010	10 kg
GranuCult® Half FRASER (Demi FRASER) Broth (Base) with antibiotics acc. ISO 11290	1.00025.0500	500 g
FRASER Listeria Ammonium Iron (III) Supplement	1.00092.0010	10 x 1 vial
Readybag® Half FRASER (Demi FRASER) Broth with Supplements acc. ISO 11290, 12,5 g, irradiated	1.02449.0060	60 x 12,5 g bags
Readybag® Half FRASER (Demi FRASER) Broth with Supplements acc. ISO 11290, 62 g, irradiated	1.01865.0001	35 x 62 g bags
ReadyTube™ 225 Half Fraser ISO 11290	1.46476.0006	6 x 225 ml
GranuCult® FRASER Broth (Base) acc. ISO 11290	1.10398.0500	500 g
FRASER Listeria Selective Supplement (antibiotics)	1.00093.0010	10 x 1 vial
ReadyTube™ 10 Fraser ISO 11290	1.46208.0020	20 x 10 ml
Chromocult® Listeria Agar (Base) acc. OTTAVIANI and AGOSTI acc. ISO 11290	1.00427.0500	500 g
Chromocult® Listeria Agar Enrichment Supplement	1.00439.0010	10 x1 vial
Chromocult® Listeria Agar Selective Supplement	1.00432.0010	10 x1 vial
ReadyPlate™ CHROM Listeria Agar ISO 11290	1.46186.0020	20 x 90 mm
GranuCult® Oxford agar (Base) acc. FDA-BAM and ref. to ISO 11290	1.07004.0500	500 g
Oxford-Listeria-Selective Supplement	1.07006.0010	10 x 1 vial
PALCAM Agar acc. VAN NETTEN et al. (Base) acc. FDA-BAM and ref. to ISO 11290	1.11755.0500	500 g
PALCAM *Listeri*a Selective-Supplement acc. VAN NETTEN et al.	1.12122.0010	10 x 1 vial