Technical Data Sheet

EcoCult®
Buffered Listeria enrichment broth (base) (BLEB) acc. FDA-BAM

Ordering number: 1.40185.5000 / 1.40185.9010

For the primary selective enrichment of *Listeria* spp. including *Listeria monocytogenes* from food and environmental samples.

Buffered Listeria enrichment broth (base) (BLEB) acc. FDA-BAM is also known as bLEB.

This culture medium complies with the specifications given by FDA-BAM Medium M52.

Mode of Action

The BLEB enrichment broth is a modification of the formulation of Tryptic Soy Broth with the addition of yeast extract and selective agents and by increasing its buffering capacity. Glucose (dextrose) is the carbohydrate source. Sodium chloride maintains the osmotic balance of the medium. Phosphate acts as a buffer. Sodium pyruvate mediates the recovery of sublethally damaged *Listeria* spp. The addition of acriflavine, cycloheximide and nalidixic acid inhibits the growth of the accompanying flora.

Acriflavine, cycloheximide and nalidixic acid are added after a 4 h pre-enrichment of the sample in BLEB broth base. This delay in addition of the selective agents is intended to promote the resuscitation and growth of injured *Listeria* spp.

Typical Composition

Specified by FDA-BAM (M52)		EcoCult® Buffered Listeria enrichment broth (base) (BLEB) acc. FDA-BAM	
Trypticase Soy broth	30 g/l	Tryptic Soy Broth* contains: Peptone from casein Peptone from soymeal D(+) Glucose NaCl K_2HPO_4	30 g/l 17.0 g/L 3.0 g/L 2.5 g/l 5.0 g/l 2.5 g/l
Yeast Extract	6. g/l	Yeast Extract	6.0 g/l
Na_2HPO_4 (anhydrous)	9.6 g/l	Na_2HPO_4 (anhydrous)	9.6 g/l
KH_2PO_4 (anhydrous)	1.35 g/l	KH_2PO_4 (anhydrous)	1.35 g/l
Sodium pyruvate (sodium salt)	1.11 g/l	Sodium pyruvate (sodium salt)	1.11 g/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	7.3 ± 0.1	pH at 25 °C	7.3 ± 0.1
Supplements added after autoclaving and after 4 h pre-enrichment of the sample:			
Acriflavin HCl	10 mg/l	Acriflavin HCl	10 mg/l
Nalidixic acid (sodium salt)	40 mg/l	Nalidixic acid (sodium salt)	40 mg/l
Cycloheximide	50 mg/l	Cycloheximide	50 mg/l

* Tryptic Soy broth is also known as Trypticase Soy Broth.

Preparation

Dissolve 24.0 g in 500 ml purified water. Dispense in 225 ml aliquots and autoclave (15 minutes at 121°C).

According FDA- BAM, the selective supplement is added to after a 4 h pre-enrichment of the sample in BLEB broth base.

Thereafter, the appropriate aliquot of supplement is added: 0.5 ml from a vial of Listeria Selective Supplement, Cat. No. 111781, reconstituted by adding of 1 ml sterile purified water.

Mix the enrichment with the supplement and continue incubation for the remainder of the enrichment period.

The dehydrated medium is a powder with light yellow colour.

The prepared medium is clear to slightly opalescent and yellowish-brown. The pH value at 25 °C is in the range of 7.2 – 7.4.

Experimental Procedure and Evaluation

Depends on the purpose for which the medium is used.

According FDA- BAM, inoculate 25 g of the sample to 225 ml broth and incubate 4 h at (30 ± 2 °C).

Thereafter, the appropriate aliquot of supplement is added: 0.5 ml from a vial of Listeria Selective Supplement, Cat. No. 111781, reconstituted by adding of 1 ml sterile purified water.

Mix the enrichment with the supplement and continue incubation at (30 ± 2° C) for the remainder of the 24 to 48 h enrichment period.

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] + *Saccharomyces cerevisiae* ATCC® 9763 [WDCM 00058]	4 h at (30 ± 1 °C) aerobically, then supplement added Incubation is continued for another (44 ± 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] + *Saccharomyces cerevisiae* ATCC® 9080				
Selectivity	*Escherichia coli* ATCC® 8739 [WDCM 00012]		Qualitative	<10 colonies on Tryptic Soy Agar (TSA)	-
	Escherichia coli ATCC® 25922 [WDCM 00013]				
	Saccharomyces cerevisiae ATCC® 9763 [WDCM 00058]			Total inhibition on Sabouraud Dextrose Agar (SDA)	-
	Saccharomyces cerevisiae ATCC® 9080				

Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with EN ISO 11133:2014.

Literature

FDA-BAM (2017) Chapter No. 10: Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods. U.S. Food and Drug Administration - Bacteriological Analytical Manual.

FDA-BAM (2018): Media Index for BAM - BAM Media M52: Buffered Listeria Enrichment Broth (BLEB). Food and Drug Administration - Bacteriological Analytical Manual.

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

Lovett, J., Francis, D.W. and Hunt, J.M. (1987): *Listeria monocytogenes* in Raw Milk: Detection, Incidence, and Pathogenicity. J. Food. Prot. **50**(3): 188-192.

Ordering Information

Product	Cat. No.	Pack size
EcoCult® Buffered Listeria enrichment broth (base) (BLEB) acc. FDA-BAM	1.40185.5000	5 kg
EcoCult® Buffered Listeria enrichment broth (base) (BLEB) acc. FDA-BAM	1.40185.9010	10 kg
Listeria Enrichment Broth (LEB) acc. to FDA/IDF-FIL (granulated medium)	1.09628.0500	500 g
Listeria Enrichment Broth (LEB) acc. to FDA/IDF-FIL (granulated medium)	1.09628.5000	5 kg
Listeria selective enrichment supplement FDA-BAM 1995 / IDF-FIL (contains Acriflavine, Cycloheximide, Nalidixic acid sodium salt)	1.11781.0010	10 x 1 vial