thermoscientific

Oxoid Dehydrated Culture Medium

***BRILLIANCE*™ LISTERIA AGAR (ISO)**

REF **CM1212; BO1370Z; PO1298A; PO5332A**

Intended Use
For professional use only by trained/skilled microbiology laboratory personnel, i.e., technicians and laboratory managers in food microbiology. To be prepared following standard laboratory media preparation processes by food chain testing Microbiology laboratories (food, animal feed and environmental samples), i.e., both service laboratories and food/animal feed producers.

Thermo Scientific™ Oxoid™ *Brilliance*™ Listeria Agar (ISO) is a selective and differential medium for the detection, enumeration and presumptive identification of *Listeria monocytogenes* and *Listeria* species from food, animal feed and environmental samples according to ISO 11290-1:2017 and ISO 11290-2:2017 standards and other national reference methods using the Ottaviani & Agosti ALOA formulation (i.e. FDA/BAM and Health Canada), and the Oxoid™ Listeria Precis™ workflow (5)(6).

Indication for Use
For analysis of food, animal feed and environmental samples according to GLP as described in the ISO 11290-series, national reference methods, and Oxoid™ Listeria Precis™ validated method. Not intended for clinical use or the diagnosis of disease.

Summary and Explanation
Listeria monocytogenes is the most common pathogenic *Listeria* species and has been shown to be pathogenic to both humans and animals. Although *L. ivanovii* is primarily pathogenic to animals, there are strains which have been shown to cause infection in humans (1). Studies have shown the ALOA medium (described by Ottaviani and Agosti) to be superior to PALCAM and Oxford medium in the isolation of *Listeria monocytogenes* (2).

Oxoid *Brilliance* Listeria Agar (ISO) uses the chromogen 5-Bromo-4-chloro-3-indolyl-ß-d-glucopyranoside (X-glucoside) for presumptive identification of *Listeria* spp. This chromogen is cleaved by ß-glucosidase, which is common to all *Listeria* species. Other organisms that possess this enzyme, such as enterococci, are inhibited by the selective agents within the medium: lithium chloride, polymyxin B, nalidixic acid and ceftazidime, whilst amphotericin B inhibits the growth of yeasts and moulds that may be present in the sample. *L. monocytogenes* and *L. ivanovii* are then further differentiated by their ability to produce the phospholipase enzymes phosphatidylinositol-specific phospholipase C (PIPLC) and phosphatidylcholine-specific phospholipase C (PCPLC) which hydrolyse phosphotidylinositol or lecithin in the medium, producing an opaque halo around the colony.

Brilliance Listeria Agar Base (ISO) CM1212 – (dehydrated culture medium) and BO1370Z (prepared medium in bottles), when supplemented with *Brilliance* Listeria Selective Supplement (ISO) (SR0257) and *Brilliance* Listeria Differential Supplement (ISO) (SR0258), and pre-prepared *Brilliance* Listeria Agar (ISO) plates (PO1298A and PO5332A), conform to the ALOA formulation which is used in ISO 11290-1:2017 for detection (3) and ISO 11290-2:2017 for enumeration (4) of *Listeria* spp. The ALOA formulation incorporates phosphatidylinositol so that PIPLC, produced by *L. monocytogenes*, is detected. Both PIPLC and PCPLC are associated with the virulence of *Listeria* and, therefore, detection of either enzyme is a useful indicator of pathogenicity.

Principle
Both enzymatic digests form the nutritional sources within the medium, whilst yeast extract provides growth promoting B vitamins. Sodium pyruvate is protective of damaged cells and promotes longevity. Glucose is a fermentable carbohydrate energy source. Magnesium glycerophosphate is a buffering agent. Magnesium sulphate provides magnesium ions, which are essential for bacterial replication. Sodium chloride maintains the osmotic equilibrium. Lithium chloride is a selective agent. X-glucoside provides the chromogen to distinguish *Listeria* species. Agar is the solidifying agent. Amphotericin B is added to suppress fungi. Nalidixic acid and polymyxin B are added to inhibit gram-negative organisms. Ceftazidime, a third-generation cephalosporin is a broad-spectrum antibiotic.

Typical Formula*	grams per litre
Enzymatic digest of animal tissues	18.0
Enzymatic digest of casein	6.0
Yeast extract	10.0
Sodium pyruvate	2.0
Glucose	2.0
Magnesium glycerophosphate	1.0
Magnesium sulphate (anhydrous)	0.5
Sodium chloride	5.0
Lithium chloride	10.0
Disodium hydrogen phosphate (anhydrous)	2.5
5-Bromo-4-chloro-3-indolyl-ß-d-glucopyranoside	0.05
Agar	12.0

*adjusted as required to meet performance standards

Physical characteristics

Appearance (CM1212)	Straw powder
Colour on reconstitution	Orange/brown
Moisture level (CM1212)	≤7%
pH	7.2 ± 0.2 at 25°C
Clarity	Clear
Gel strength	Firm, comparable to 12.0g/l of agar
Fill weight (BO1370Z)	206.0 ± 2g (200ml)
Fill weight (PO1298A)	19.0g ± 2g
Fill weight (PO5332A)	17.5g ± 5%

Supplementation
***Brilliance* Listeria Selective Supplement SR0257**

Vial contents	Per litre
Nalidixic acid sodium salt	20mg
Polymyxin B sulphate	76,700IU
Ceftazidime	20mg
Amphotericin B	10mg

***Brilliance* Listeria Differential Supplement SR0258**

Vial contents	Per litre
L-α-phosphotidylinositol solution	30ml

Precautions
This product should only be used by trained individuals. This includes the safe disposal of used or unused reagents and as well as any other contaminated or potentially contaminated material. It is the responsibility of each laboratory to manage waste produced according to any federal, state and local applicable regulations.

Storage
Store the dehydrated medium (CM1212) at 10-30°C away from light and ensure pots are tightly closed after use. Store prepared medium for up to 2 weeks at 2-8°C, away from light.

Store the selective (SR0257) and differential supplements (SR0258) at 2-8°C away from light. Do not freeze the differential supplement solution.

Store agar bottles (BO1370Z) at 2-8°C, away from light.

Store pre-prepared media plates (PO1298A or PO5332A) at 2-12°C away from light.

Ensure all media are used before the expiry date on the label.

Sample Collection, Handling and Storage

Samples should be collected and handled following the recommended guidelines.

Materials Required but Not Supplied
(1). Laboratory equipment as required
(2). Ancillary reagents and culture media
(3). Quality control organisms as required

Directions for preparation of CM1212 dehydrated culture medium and supplements
Suspend 34.5g in 480ml of distilled water. Mix well and sterilize by autoclaving at 121°C for 15 minutes. Cool to 48 ± 2°C. Aseptically add the contents of 1 vial of *Brilliance* Listeria Selective Supplement (ISO) (SR0257E) reconstituted as directed, and 1 vial of *Brilliance* Listeria Differential Supplement (ISO) (SR0258E) warmed to 48± 2°C. Mix well and pour into sterile Petri dishes.

Directions for preparation of BO1370Z agar bottles & supplements
Place the agar bottles in a steamer for approximately 40-45 minutes until completely melted, allow to cool slightly and transfer the molten agar to a water bath at 45 ± 1°C. Aseptically supplement each bottle with 1 vial of SR0257B (*Brilliance* Listeria Selective supplement (ISO)), reconstituted as directed and 1 vial of SR0258B [*Brilliance* Listeria Differential supplement (ISO)] warmed to 46°C and gently mixed before addition. Thoroughly mix the supplements with the molten agar by capping the bottle and gently inverting 2 or 3 times until the colour is homogenous. After melting and/or supplementation, the medium can be kept in the water bath at 45 ± 1°C for up to 4h (mixing well before use) after which time the medium must be discarded.

Technique
Brilliance Listeria Agar (ISO) can be used following the ISO 11290 protocol or the Listeria Precis workflows:

To determine the presence of absence of *L. monocytogenes* and other *Listeria* spp. in a specific volume or weight of a food or environmental sample, the following enrichment and detection method is a summary of the ISO 11290-1:2017 protocol:

1. Add a 25g food sample to 225ml of Half Fraser broth (Fraser broth base CM0895 supplemented with Half Fraser Supplement SR0166) and stomach for a minimum of 30 seconds to mix the sample.
2. Incubate the broth without agitation at 30 ± 1°C for 25 ± 1h.
3. Gently agitate the bag then, using a microbiological loop inoculate onto *Brilliance* Listeria Agar (ISO) and a second selective medium (e.g., PALCAM Agar - CM0877 & SR0150). Incubate at 37 ± 1°C for 24 ± 2h, and if necessary, for an additional 24h ± 2h (as directed by the manufacturer).
4. Examine the PALCAM plate for black colonies and the *Brilliance* Listeria Agar (ISO) plate for blue-green colonies with and without halos.
5. From the same incubated Half Fraser Broth remove 0.1ml and inoculate into 10ml of Fraser Broth CM0895 supplemented with SR0156. Incubate at 37 ± 1°C for 24 ± 2h and then repeat Steps 3 & 4 followed by step 6.
6. Confirm presumptive colonies on the agar plates as *L. monocytogenes* or *Listeria* spp. by appropriate methods - refer to ISO 11290-1:2017 (3).

To determine the number of *L. monocytogenes* and other *Listeria* spp. per gram or ml of food or environmental sample, the following enumeration method is a summary of the ISO 11290-2:2017 protocol:-

1. Add a 1:10 suspension of the sample into Buffered Peptone Water (ISO) (CM1049 or CM1211). If detection and enumeration procedures are to be carried out together, Half Fraser broth (with selective agents added to the suspension preferentially after enumeration) can be used as the diluent. For certain products prepare and dilute the sample according to the specifications of the standard ISO 6887-1:2017 (7).
2. L-spread 0.1ml of the initial suspension onto the surface of a *Brilliance* Listeria Agar (ISO) plate (90mm), and 0.1ml of further decimal dilutions onto separate plates if required.

For enumeration of low counts, the limit of detection can be increased by a factor of 10 by distributing 1ml of the initial suspension over the surface of three 90mm plates or one 140mm plate, dried beforehand, if required, in the incubator.

*Alternatively, as part of the Listeria Precis workflow, transfer 1mL of the sample preparation into a 90mm sterile petri dish and pour 20±2ml of molten (45±1°C) agar (prepared using the CM1212 dehydrated medium or BO1370Z pre-prepared bottled agar) into the petri dish.

3. Incubate at 37 ± 1°C for 24 ± 2h, and if necessary, for an additional 24 ± 2h.
4. Confirm presumptive *L. monocytogenes* and/or *Listeria* spp. colonies by appropriate methods – refer to ISO 11290-2:2017 (4).

For *Listeria Precis workflows, refer to Listeria Precis Instructions for Use: MAN0026538 (5) and the AFNOR validation website (6).

Quality Control

QC testing criteria varies according to the medium format. Please refer to the CoA's for the specific testing details.

Control Media: Tryptone Soya Agar (TSA), Columbia Blood Agar (CBA) Base enriched with 5% v/v horse blood or Sabouraud Dextrose Agar (SDA), where appropriate

Growth characteristics (24 ± 2h incubation at 37 ± 2°C)
Medium challenged with 30-120 colony-forming units

Listeria monocytogenes	NCTC 11994	0.5-2mm blue-green colonies, with halo
Listeria monocytogenes	ATCC® 7644	0.5-2mm blue-green colonies, with halo
Listeria monocytogenes	ATCC® 13932	0.5-2mm blue-green colonies, with halo

A satisfactory result is represented by recovery of positive strains equal to or greater than 50% of the control medium.

Growth characteristics (48 ± 4h incubation at 37 ± 2°C)
Medium challenged with 30-120 colony-forming units

Listeria monocytogenes	NCTC 11994	1-3mm blue-green colonies, with halo
Listeria monocytogenes	ATCC® 7644	1-3mm blue-green colonies, with halo
Listeria ivanovii	NCTC 12701	0.5-3mm blue-green colonies, with halo

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium. For *Listeria ivanovii* NCTC 12701, a satisfactory result is represented by recovery equal to or greater than 50% of the control medium.

Medium challenged with 1E+04 to 1E+05 colony-forming units

Bacillus cereus	ATCC® 10876	No growth or 1-2mm cream/blue colonies
Staphylococcus aureus	ATCC® 25923	No growth or 0.5-1mm yellow colonies
Saccharomyces cerevisiae	ATCC® 9763	No growth or 1-2mm cream/blue colonies

Negative strains are inhibited or shall produce at least a 2 $\log_{(10)}$ reduction when compared to the control medium.

Medium challenged with 1E+04 to 1E+06 colony-forming units

Proteus mirabilis	NCTC 10975	No growth

Negative strains are inhibited.

Testing performed in accordance with ISO11133:2014 (8)
Tested with the addition of SR0257 and SR0258
Growth characteristics (48 ± 4h incubation at 37 ± 2°C)
Medium challenged with 50-120 colony-forming units

Listeria monocytogenes	ATCC® 13932 WDCM 00021	1-3mm blue-green colonies, with halo
Listeria monocytogenes	ATCC® 35152 WDCM 00109	1-3mm blue-green colonies, with halo

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the TSA control medium

Medium challenged with 1E+03 to 1E+04 colony-forming units

Listeria innocua	ATCC® 33090 WDCM 00017	0.5-3mm blue-green colonies, without halo

A satisfactory result is represented by good growth with a negative diagnostic reaction.

Medium challenged with 1E+04 to 1E+06 colony-forming units

Escherichia coli	ATCC® 25922 WDCM 00013	No growth
Escherichia coli	ATCC® 8739 WDCM 00012	No growth
Enterococcus faecalis	ATCC® 29212 WDCM 00087	No growth
Enterococcus faecalis	ATCC® 19433 WDCM 00009	No growth

Negative strains are inhibited.

Note:
It is the responsibility of the user to perform Quality Control testing taking into account the intended use of the medium, and in accordance with any local applicable regulations (frequency, number of strains, incubation temperature etc.)

Performance
Performance was evaluated using fourteen bacterial strains including the following; *Listeria monocytogenes, Listeria ivanovii, Listeria innocua, Enterococcus faecalis, Escherichia coli, Bacillus cereus* and *Staphylococcus aureus*. All organisms gave expected growth characteristics according to the current product specification.

Limitations
It should be noted that, as with all media, atypical organisms may give anomalous reactions. Identifications are presumptive and should be confirmed using appropriate methods. Some strains of *L. monocytogenes* may show a very weak halo (or even no halo) when stressed. Some are also characterized by slow PIPLC activity; a longer incubation period may be required for example more than four days. Organisms, other than *Listeria* that are resistant to the antimicrobials present in the medium may be able to grow and may be able to produce blue colonies such as some strains of *Bacillus* spp., staphylococci and streptococci. Some strains of the target organisms which have particular growth requirements or sensitivity to the selective agents may grow weakly or not at all on this medium.

Packaging

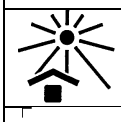
Product code	Suffix	Pack size
CM1212	B	0.5 KG
	T	5 KG
SR0257	B	10 vials to supplement 200ml of base (i.e., BO1370Z)
	E	10 vials to supplement 500ml of agar base
SR0258	B	10 vials to supplement 200ml of agar base (i.e., BO1370Z)
	E	10 vials to supplement 500ml of base
BO1370Z	10 x 200ml agar bottles (unsupplemented)	
PO1298A	10 plates wrapped in film	
PO5332A	10 plates wrapped in film	

Waste disposal
For waste disposal refer to the relevant Material Safety Data Sheet.

Bibliography

(1) Guillet C, Join-Lambert O, Le Monnier A, Leclercq A, Mechaï F, Mamzer-Bruneel MF, Bielecka MK, Scortti M, Disson O, Berche P, Vazquez-Boland J, Lortholary O, Lecuit M. Human listeriosis caused by Listeria ivanovii. Emerg Infect Dis. 2010 Jan;16(1):136-8.
(2) Vlaemynck G, Lafarge V, Scotter S. Improvement of the detection of Listeria monocytogenes by the application of ALOA, a diagnostic, chromogenic isolation medium. J Appl Microbiol. 2000 Mar;88(3):430-41.
(3) ISO 11290-1:2017 (Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and other *Listeria spp.* - Part 1: Detection method).
(4) ISO 11290-2:2017 (Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and other *Listeria spp.* - Part 2: Enumeration method).
(5) MAN0026538: see the www.thermofisher.com website or contact Technical Support.
(6) AFNOR Validation documents: nf-validation.afnor.org/en/food-industry/
(7) ISO 6887-1:2017 (Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination).
(8) ISO 11133:2014 (Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media).

Symbol Legend

Symbol	Meaning
	Catalogue number
	Manufacturer
	Temperature limitation (storage temp.)
	Use by (expiration date)
	Lot number
	Protect from light
	Consult instructions for use

thermoscientific

The ATCC Licensed Derivative® Emblem, the ATCC Licensed Derivative® word mark, and the ATCC catalog marks are trademarks of ATCC. Thermo Fisher Scientific Inc. is licensed to use these trademarks and to sell products derived from ATCC® cultures.

Oxoid Ltd, Wade Road, Basingstoke, Hants RG24 8PW, UK
(CM1212, SR0257, SR0258, PO1298A, BO1370Z)

Oxoid Deutschland GmbH, Am Lippeglacis 4-8, D-46483
Wesel (PO5332A)