thermoscientific

Product Specification Sheet

Brilliance™ Listeria Agar (ISO)

Intended Usage: A medium for the detection, enumeration and presumptive identification of *Listeria monocytogenes* and *Listeria* species from food, feed and environmental samples according to ISO 11290-1:2017 and ISO 11290-2:2017 standards and other national reference methods using Ottaviani & Agosti formulation (i.e. FDA/BAM and Health Canada).

For professional use only.

	PO5332A
Version: 01	Revision Date: 29 April 2022

Thermo Scientific™ Brilliance™ Listeria Agar (ISO)

Form of Product	Poured plate
Storage	2 – 12°C, dark
Filling weight	17.5 g ± 5 %
Packaging	10 plates wrapped in film
pH	7.2 ± 0.2
Appearance	Honey yellow, translucent
Shelf life	10 weeks
Intended Usage	A medium for the detection, enumeration and presumptive identification of *Listeria monocytogenes* and *Listeria* species from food, feed and environmental samples according to ISO 11290-1:2017 and ISO 11290-2:2017 standards and other national reference methods using Ottaviani & Agosti formulation (i.e. FDA/BAM and Health Canada). For professional use only.
Technique	Depends on the different methods. For information see IFU for Thermo Scientific™ Oxoid™ CM1212 / SR0257 / SR0258

Typical formulation*	g/l
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
Di-sodium hydrogen phosphate (anhydrous)	2.5
5-Bromo-4-chloro-3-indolyl-β-D-glucopyranoside	0.05
Agar	12.0
Nalidixic acid sodium salt	0.02
Polymyxin B sulphate	76700 IU
Ceftazidime	0.02

Amphotericin B	0.01
L-α-phosphatidylinositol	2.0

*Adjusted as required to meet performance standards.

Quality Control

1. Control for general characteristics, labelling and printing.

2. Contamination check
   ≥ 72 h @ 20 – 25 °C, aerobic
   ≥ 72 h @ 30 – 35 °C, aerobic

3. Microbiological control

Positive Controls	Growth
Inoculum 50 – 120 colony forming units (cfu), quantitative **Incubation conditions: 22 – 26 h @ 36 ± 1°C, aerobic**	
Listeria monocytogenes ATCC® 13932 (WDCM 00021)	0.5-2.0 mm, blue-green colonies with halo
Listeria monocytogenes NCTC 11994 (WDCM 00019)	0.5-2.0 mm, blue-green colonies with halo
Colony counts shall be ≥ 50% of the control medium (TSA)	

Positive Controls	Growth
Inoculum 50 – 120 colony forming units (cfu), quantitative **Incubation conditions: 44 – 52 h @ 36 ± 1°C, aerobic**	
Listeria monocytogenes ATCC® 13932 (WDCM 00021)	1.0-3.0 mm, blue-green colonies with halo
Listeria monocytogenes NCTC 11994 (WDCM 00019)	1.0-3.0 mm, blue-green colonies with halo
Listeria monocytogenes ATCC® 35152 (WDCM 00109)	1.0-3.0 mm, blue-green colonies with halo
Colony counts shall be ≥ 50% of the control medium (TSA)	

Specificity Control	Growth
Inoculum 10^3 – 10^4 cfu, qualitative, control medium COL+SB Incubation conditions: 44 – 52 h @ 36 ± 1°C, aerobic	
Listeria innocua ATCC® 33090 (WDCM 00017)	Good growth, Blue-green colonies, no halo

Negative Controls	Growth
Inoculum ≥ 10^4 cfu, quantitative, control medium TSA Incubation conditions: 44 – 52 h @ 36 ± 1°C, aerobic	
Escherichia coli ATCC® 25922 (WDCM 00013)	Complete inhibition
Enterococcus faecalis ATCC® 29212 (WDCM 00087)	Complete inhibition

Tested in accordance with ISO 11133:2014
The formulation of this medium conforms to EN ISO 11290-1 and EN ISO 11290-2.

ATCC® registered trademark of American Type Culture Collection.

Description

Thermo Scientific™ 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.

Technique

Brilliance Listeria Agar (ISO) can be used following the ISO 11290 protocol:

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 25g of 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 30ºC ± 1°C for 25h ± 1h hours.
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 37ºC ± 1°C for 24h ± 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 37ºC ± 1°C for 24h ± 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 (**1**).

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 can be used as the diluent. For certain products prepare and dilute the sample according to the specifications of the standard ISO 6887.

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.

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

Literature

1. 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).

2. 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).