

Technical Data Sheet

Chromocult®

Listeria Agar (Base) acc. OTTAVIANI and AGOSTI acc. ISO 11290

Ordering number: 1.00427.0500

For the isolation and differentiation of *Listeria monocytogenes* and other *Listeria spp.* from food and animal feed, environmental samples in the area of food production and food handling and other materials.

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

Mode of Action

This medium contains the chromogenic compound 5-bromo-4-chloro-3-indolyl- β -D-glucopyranoside, a substrate for the detection of β -glucosidase. This enzyme is common in all *Listeria*, these appear on the medium as blue-green colored colonies.

Differentiation of *Listeria monocytogenes* from other *Listeria spp.* is achieved through the production of a phosphatidylinositol-specific phospholipase C (PI-PLC). *Listeria monocytogenes* hydrolyses the specific purified substrate added to the medium producing an opaque halo around the colonies.

Most *Listeria ivanovii* also produce an opaque halo around the colonies after 48 h incubation.

This medium contains a basis which ensures good growth for a broad range of bacteria. The addition of inhibitors results in a marked reduction in the growth of the majority of concomitant gram-positive and gram-negative pathogens, as well as of yeasts and fungi. Selectivity is obtained by the addition of lithium chloride, nalidixic acid and cycloheximide, whilst agar is the solidifying agent.

Typical Composition

Specified by ISO 11290		Chromocult® <i>Listeria</i> Agar acc. OTTAVIANI and AGOSTI acc. ISO 11290	
Enzymatic Digest of Animal Tissues	18 g/l	Enzymatic Digest of Animal Tissues	18 g/l
Enzymatic Digest of Casein	6 g/l	Enzymatic Digest of Casein	6 g/l
Yeast Extract	10 g/l	Yeast Extract	10 g/l
Sodium Pyruvate	2 g/l	Sodium Pyruvate	2 g/l
Glucose	2 g/l	Glucose	2 g/l
Magnesium Glycerophosphate	1 g/l	Magnesium Glycerophosphate	1 g/l
MgSO ₄ , anhydrous	0.5 g/l	MgSO ₄ , anhydrous	0.5 g/l
NaCl	5 g/l	NaCl	5 g/l
LiCl	10 g/l	LiCl	10 g/l
Na ₂ HPO ₄ , anhydrous	2.5 g/l	Na ₂ HPO ₄ , anhydrous	2.5 g/l
5-Bromo-4-Chloro-3-Indolyl-β-D-Glucopyranoside	0.05 g/l	5-Bromo-4-Chloro-3-Indolyl-β-D-Glucopyranoside	0.05 g/l
Agar	12-18 g/l	Agar-Agar*	13 g/l
Supplements added after heating			
Nalidixic Acid Sodium Salt	0.02 g/l	Nalidixic Acid Sodium Salt	0.02 g/l
Ceftazidime	0.02 g/l	Ceftazidime	0.02 g/l
Polymyxin B Sulfate	76700 IU	Polymyxin B Sulfate	76700 IU
Amphotericin B	0.01 g/l	Amphotericin B	0.01 g/l
L-α-Phosphatidylinositol	2 g/l	L-α-Phosphatidylinositol	2 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

* Agar-Agar is equivalent to other different terms of agar.

Preparation

Dissolve 35 g of base medium in a total of 476 ml of purified water. For this, partially dissolve the medium with 300 ml of water while swirling. Subsequently add the remaining 176 ml, mix thoroughly, dissolve in boiling water, and agitate frequently until completely dissolved.

Then cool the medium in a water bath to 47-50 °C.

Add the contents of one vial of Selective Supplement (article number 1.00432.0010) and distribute homogeneously.

Subsequently mix in the contents of one vial of Enrichment Supplement (article number 1.00439.0010) that has been pre-heated to 47-50 °C.

The antibiotic solution is sensitive to heat. Directly after adding the antibiotic and enrichment solution pour plates up to a volume of 15-20 ml in dishes with a diameter of 90 mm.

The prepared medium is slightly opalescent to opalescent and yellowish.

There should be no visible moisture on the plates before use. When moisture is present, the plates should be dried for the minimum time required to remove visible moisture, following the procedure as described by EN ISO 11133.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by EN ISO 11290-1, inoculate the surface of the medium from the primary and secondary selective enriched cultures so that well-isolated colonies will be obtained.

Following the procedure given by EN ISO 11290-2, inoculate the surface of the medium direct with the initial suspension of the sample so that well-isolated colonies will be obtained.

Incubate the inoculated plates inverted under aerobic conditions, e.g. acc. to EN ISO 11290 at 36-38 °C for 21-27 h, and if necessary after a further 21-27 h.

Examine the plates after the incubation for 21-27 h (and for an additional 21-27 h if the growth is weak or if no colonies is observed after 24 h incubation), examine the plates for the presence of colonies presumed to be *Listeria spp.*

Consider as *L. monocytogenes* the green-blue colonies surrounded by an opaque halo (typical colonies). If growth is slight, or if no colony is observed, or if no typical colony is present after 21-27 h incubation, re-incubate the plates for further 21-27 h and examine again.

This presumptive evidence must be confirmed by carrying out the usual tests, e.g. those described by EN ISO 11290.

Some strains of *L. monocytogenes* show a very weak halo (even no halo) in cases of stress, in particular acid stress. Some rare *L. monocytogenes* are characterized by a slow PI-PLC (phosphatidyl inositol phospholipase C) activity. Such bacteria are detected when the total duration of incubation is more than, for example, 4 days; some of these strains could be pathogenic (Leclercq, 2004).

No *L. monocytogenes* strains have been described as PI-PLC negative.

Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

According Corry et al. (2012 self-prepared plates can be stored at +2 °C to +8 °C in the dark and protected against evaporation at least four weeks

Quality Control

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	<i>Listeria monocytogenes</i> 1/2a ATCC® 35152	40-48 h at 36-38 °C	Tryptic Soy Agar (TSA)	Quantitative	Recovery ≥50 %, blue green colonies with opaque halo
	<i>Listeria monocytogenes</i> 4b ATCC® 13932				
Selectivity	<i>Escherichia coli</i> ATCC® 8739	40-48 h at 36-38 °C	-	Qualitative	Total inhibition
	<i>Escherichia coli</i> ATCC® 25922				
	<i>Enterococcus faecalis</i> ATCC® 19433				
	<i>Enterococcus faecalis</i> ATCC® 29212				
Specificity	<i>Listeria innocua</i> ATCC® 33090	40-48 h at 36-38 °C	-	Qualitative	No recovery limit specified, blue green colonies without opaque halo

Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of EN ISO 11133. A recovery rate of 50 % is equivalent to a productivity value of 0.5.



Listeria monocytogenes
ATCC® 13932



Listeria innocua
ATCC® 33090

Literature

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

Bauwens, L., Vercammen, F. and Hensens, A. (2003): Detection of pathogenic *Listeria spp.* in zoo animal faeces: use of immunomagnetic separation and a chromogenic isolation medium. *Vet. Microbiol.* **91**: 115 - 123.

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. 658-662. Royal Society of Chemistry, Cambridge, UK.

FDA-BAM (2013): Chapter No. 10: Detection and Enumeration of *Listeria monocytogenes* in Foods. U.S. Food and Drug Administration - Bacteriological Analytical Manual.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method -- Amendment 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data. EN ISO 11290-1:1998 + Amd 1:2004.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method - Amendment 1: Modification of the enumeration medium. EN ISO 11290-2:1998 + Amd 1:2004.

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

Leclercq, A. (2004): Colonial atypical morphology and low recoveries of *Listeria monocytogenes* strains on Oxford, PALCAM, Rapid'L.mono and ALOA solid media. *J. Microbiol. Methods.* **57**: 251-258.

Notermans, S.H.W., Dufrenne, J., Leimeister-Wachter, M., Domann, E. and Chakrabony, T. (1991): Phosphatidylinositol-specific phospholipase C activity as a marker to distinguish between pathogenic and non-pathogenic *Listeria species*. *Appl. Environ. Microbiol.* **57**: 2666 - 2670.

Ottaviani, E.; Ottaviani, M. and Agosti, M. (1997): Differential agar medium for *Listeria monocytogenes*. *Ind. Aliment.* **36**: 888.

Vlaemynck, G., Lafarge, V. and Scotter, S. (2000): Improvement of the detection of *Listeria* by the application of ALOA, a diagnostic, chromogenic isolation medium. *J. Appl. Microbiol.* **88**: 430 - 441.

Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
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	100 x 90 mm
GranuCult™ Half FRASER (Demi FRASER) Broth (Base) with Antibiotics acc. ISO 11290	1.00025.0500	500 g	
Readybag® Half FRASER (Demi FRASER) Broth with Supplements acc. ISO 11290, 12.5 g, irradiated	1.02449.0060	60 bags	35 x 62 g bags
ReadyTube™ 2000 Half Fraser ISO 11290	1.46646.0001	2000 ml bag	6 x 225 ml
GranuCult™ FRASER Broth (Base) acc. ISO 11290	1.10398.0500	500 g	
FRASER Listeria Selective Supplement	1.00093.0010	10 x 1 vial	
FRASER Listeria Ammonium Iron (III) Supplement	1.00092.0010	10 x1 vial	
ReadyTube™ 10 Fraser ISO 11290	1.46208.0020	20 x 10 ml	100 x 10 ml
Oxford-Listeria-Selective Agar (Base)	1.07004.0500	500 g	
Oxford-Listeria-Selective Supplement	1.07006.0010	10 x 1 vial	
Oxford Listeria Selective Agar	1.46328.0020	20 pcs	
PALCAM Listeria-Selective Agar (Base) acc. to VAN NETTEN et al.	1.11755.0500	500 g	
PALCAM Listeria Selective-Supplement acc to van Netten et al	1.12122.0010	10 x 1 vial	
PALCAM Listeria Selective Agar	1.46329.0020	20 pcs	
L-PALCAM-Listeria Selective Enrichment Broth (Base) acc to van Netten et al.	1.10823.0500	500 g	
Singlepath® Listeria	1.04142.0001	25 tests	
Singlepath® L' mono	1.04148.0001	25 tests	
ReadyTube™ 9 BPW ISO 6579, 6887, 21528	1.46142.0020	20 x 9 ml	100 x 9 ml, 6 x 225 ml, 6 x 1000 ml, 1 x 2000 ml

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