Technical Data Sheet

ReadyPlate™ CHROM Listeria Agar acc. OTTAVIANI and AGOSTI acc. ISO 11290

Ordering number: 1.46186.0020 / 1.46186.0100

For 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 phopspholipase 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		ReadyPlate™ CHROM Listeria Agar	
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_2HPO_4, anhydrous	2.5 g/l	Na_2HPO_4, anhydrous	2.5 g/l
5-Bromo-4-Chloro-3-Indolyl-ß-D-Glucopyranoside	0.05 g/l	5-Bromo-4-Chloro-3-Indolyl-ß-D-Glucopyranoside	0.05 g/l
Naladixic Acid Sodium Salt	0.02 g/l	Naladixic 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		Amphotericin	0.01 g/l
L-α-Phosphatidylinositol	2 g/l	Soy Lecithin*	2 g/l
Agar	12-18 g/l	Agar	15 g/l
Water	1000 ml/l	Water	1000 ml/l
pH at 25 °C	7.2 ± 0.2	pH at 25 °C	7.2 ± 0.2

* ISO DIS 11290 states that 2 g of Soy Lecithin can be used as replacement for L-α-phosphatidylinositol
but must contain 9-15 % unfractionated phosphatidylinositol

Application and Interpretation

Depending on the purpose for which the medium is used.

Each plate is provided with a label including a data matrix code for paperless plate identification. The code consists of a two-dimensional 20-digit serial number, which harbors the following information:

digits 1-3: here code 767 (corresponds to article 146186); digits 4-9: lot number; digits 10-14: batch specific individual number; digits 15-20: expiration date (YY/MM/DD).

Please check each agar plate before using it on sterility and pay attention to aseptic handling in order to avoid false positive results.

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 *Listeria monocytogenes* strains have been described as PI-PLC negative.

Storage and Shelf Life

The product can be used for sampling until the expiry date if stored upright, protected from light and properly sealed at +2 °C to +8 °C.

Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	*Listeria monocytogenes* 4b ATCC® 13932	40-48 h at 36-38 °C	Tryptic Soy Agar (TSA)	Quantitative	Recovery ≥ 50 %, blue green colonies with opaque halo
	Listeria monocytogenes 1/2a, 3a DSM 112143				
Selectivity	*Escherichia coli* ATCC® 8739	40-48 h at 36-38 °C	Tryptic Soy Agar (TSA)	Qualitative	Total inhibition
	Escherichia coli ATCC® 25922				
	Enterococcus faecalis ATCC® 29212				

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Specificity	*Listeria innocua* ATCC® 33090	40-48 h at 36-38 °C	Tryptic Soy Agar (TSA)	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.

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
ReadyPlate™ CHROM Listeria Agar ISO 11290	1.46186.0020	20 x 90 mm	100 x 90 mm
ReadyTube™ 225 Half Fraser ISO 11290	1.46476.0001	6 x 225 ml	
ReadyTube™10 Fraser ISO 11290	1.46208.0020	20 x 10 ml	100 x 10 ml
GranuCult™ Half Fraser Broth (Base) with Antibiotics ISO 11290	1.00025.0500	500 g	
GranuCult™ Fraser Broth (Base) with Antibiotics 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 x 1 vial	
Chromocult® Listeria Agar Enrichment Supplement	1.00439.0010	10 x 1 vial	
Chromocult® Listeria Agar Selective Supplement	1.00432.0010	10 x 1 vial	
Chromocult® Listeria Agar (Base) acc OTTAVIANI and AGOSTI ISO 11290	1.00427.0500	500 g	
ReadyTube™ 9 BPW ISO 6579, 6887, 21528	1.46142.0020	20 x 9ml	6 x 225 ml, 6 x 1000 ml,

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