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Chromocult® Listeria Selective Agar Base

Selective Agar for the detection and enumeration of Listeria monocytogenes in foods

MERCK

Chromocult® Listeria Selective Agar Base

Ord. No. 1.00427.0500

acc. to OTTAVIANI and AGOSTI, (ISO 11290)

Culture medium for the detection and enumeration of *Listeria monocytogenes* in foods.

The culture medium corresponds to Agar Listeria acc. to Ottaviani and Agosti in line with the recommendations of ISO 11290 (2004) and FDA/BAM (2003).

Mode of action

The rich basis of Chromocult® Listeria Selective Agar 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.

L. monocytogenes and *L. innocua* show virtually uninhibited growth, whilst the growth of other listeriae may be retarded *(L. ivanovii)* or inhibited *(L. seeligeri)*.

The addition of 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside makes it possible to differentiate between β-D-glucosidase-positive and negative bacteria.

Listeriae are β-D-glucosidase-positive and grow on the medium in the form of blue-green colonies.

To detect *L. monocytogenes* L-α-phosphatidylinositol is added to the medium. *L. monocytogenes* has the enzyme phosphatidylinositol phospholipase C (PI-PLC) described as a virulence factor. This phospholipase activity results in the formation of opaque haloes around *L. monocytogenes* colonies.

Apart from *L. monocytogenes*, only *L. ivanovii* among the listeriae shows phospholipase C activity.

Typical composition (g/litre)

Peptone from meat 18.0; peptone from casein 6.0; yeast extract 10.0; sodium pyruvate 2.0; glucose 2.0; magnesium glycerophosphate 1.0; magnesium sulphate 0.5; sodium chloride 5.0; lithium chloride 10.0; disodium hydrogen phosphate 2.5; 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside; agar-agar 13.0.

Preparation

1. 35 g base medium are completely dissolved in a total of 476 ml demineralised water. First partially dissolve the dry medium with 300 ml water using a swirling motion. Then add the remaining 176 ml water, mix thoroughly and dissolve completely in a boiling water bath (steamer), swirling occasionally (approx. 20–35 min).
   Do not autoclave – Do not overheat.
2. After it has dissolved completely, the medium is cooled to 50–48 °C in the water bath.
3. The contents of a bottle of Selective Supplement are mixed with 4 ml sterile demineralised water/ethanol mixture (1:1) under aseptic conditions. The supplement (yellow suspension) is added to the nutrient medium solution and mixed by swirling to render it homogeneous.
4. A bottle of the sterile Enrichment Supplement (20 ml) is heated to 50–48 °C and mixed into the base medium solution under aseptic conditions. Stir gently during this addition.

The antibiotic solution is sensitive to heat. Poor plates directly after adding the antibiotic and enrichment solution (16–18 ml per Petri dish).

pH: 7.2 ± 0.2 at 25 °C.

The prepared medium has a uniform appearance, slightly cloudy and yellowish in colour.

The prepared plates can be kept for 4 weeks (+2 °C to +8 °C) under suitable storage conditions - protected from drying out and light.

Application and interpretation

- Bring the plates up to room temperature.
- If condensation occurs, dry plates immediately before use (approx. 20 min. at 55 °C).
- Inoculation after enrichment (ISO-11290-1 detection method)

First enrichment

The first enrichment is performed in 1/2 Fraser broth (dilution 1:10, e.g. 25 g sample in 225 ml 1/2 Fraser broth). The 1/2 Fraser broth is incubated at 30 °C for 24 ± 3 hours.

Using an inoculating loop, a sample is taken from the first enrichment and spread on the Chromocult® Listeria Selective Agar to produce clearly isolated single colonies.

Second enrichment

10 ml Fraser broth are inoculated with 0.1 ml of the first enrichment (1/2 Fraser broth). The Fraser broth is incubated at 35 °C or 37 °C for 48 ± 3 hours.

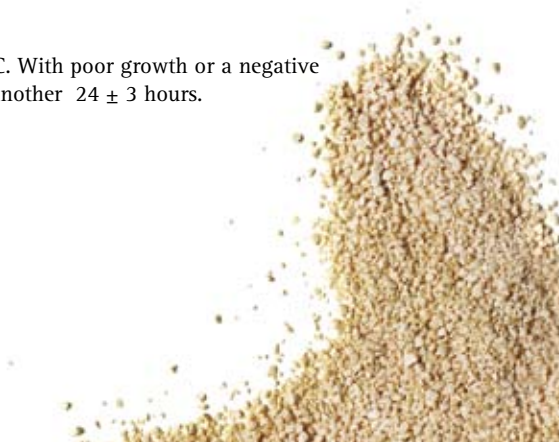
Using an inoculating loop, a sample is taken from the second enrichment and spread on the Chromocult® Listeria Selective Agar to produce clearly isolated single colonies.

Inoculation direct from food sample (ISO-11290-2 enumeration method)

The sample is diluted in buffered peptone water (1:10 and further decimal increments). The surface-culture process is used to remove 0.1 ml of the sample homogenate and the corresponding dilutions.

Incubation

24 ± 3 hours at 37 °C. With poor growth or a negative result, incubate for another 24 ± 3 hours.



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Colour makes the difference.

Interpretation

All colonies which appear blue-green with an opaque halo on the medium are counted as suspect *L. monocytogenes* colonies (typical colonies).

The suspect colonies must be confirmed, as *L. ivanovii* and some other bacteria, e.g. bacilli, may show the typical colony pattern of *L. monocytogenes*.

Quality control

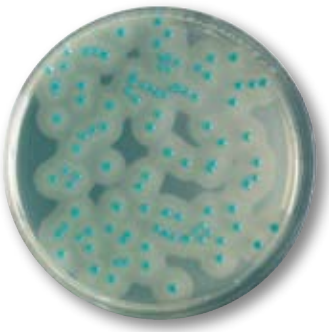
Test strains	Percentage recovery	Colony colour
Listeria monocytogenes ATCC 13932	> 50 %	blue-green with opaque halo
Listeria monocytogenes ATCC 19111	> 50 %	blue-green with opaque halo
Listeria innocua ATCC 33090	> 50 %	blue-green
Escherichia coli ATCC 25922	< 0,001 %	-
Enterococcus faecalis ATCC 19433	< 0,001 %	-
Candida tropicalis ATCC 750	< 0,001 %	-

Storage

Can be used until the expiry date if stored dry and tightly closed at room temperature (15–25 °C).

Ordering information

Merck Ord. No.	Product
1.00427.0500	Chromocult® Listeria Selective Agar, Base, acc. Ottaviani und Agosti
1.00432.0010	Listeria Agar Selective Supplement
1.00439.0010	Listeria Agar Enrichment Supplement
1.07228.0500	Peptone water, buffered
1.10398.0500	Fraser Listeria Selective Enrichment Broth (base)
1.10399.0001	Fraser Listeria Selective Supplement (2 x 8 phials, for preparation of FRASER broth)
1.04148.0001	Singlepath® L`mono



Listeria monocytogenes ATCC 13932

Confirmation of the isolated pathogens can be performed using Singlepath® L`mono, for example.

Listeria innocua ATCC 33090

Literature

Notermans, S.H.W., Dufrenne, J., Leimeister-Wächter, M., Domann, E., and Chakraborty, T. 1991. Phosphatidylinositol-specific phospholipase C activity as a marker to distinguish between pathogenic and nonpathogenic Listeria species. Appl. Environ. Microbiol. 57:2666 – 2670.

Ottaviani, E.; Ottaviani, M., and Agosti, M. 1997. Differential agar medium for Listeria monocytogenes. Industrie Alimentari 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.

Bauwens, L., Vercammen, F., and Hertsens, 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.

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. ISO 11290:2004.

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. ISO 11290:2004.

FDA/BAM – Detection and Enumeration of Listeria monocytogenes Chapter 10 (January 2003)

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