

Harlequin® *Listeria* Chromogenic Agar (Ottaviani & Agosti) (NCM1004)

Intended Use

Listeria Chromogenic Agar (according to the formulation of Ottaviani and Agosti) is a selective medium for the isolation and presumptive identification of *Listeria monocytogenes* from foodstuffs and related materials as described in ISO 11290-1:2017. This medium is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Lithium chloride in the base medium and supplementary antimicrobial compounds Ceftazidime, Polymyxin, Nalidixic acid and Cycloheximide provide the medium's selectivity. Chromogenic activity is as a result of a chromogenic substrate for the detection of the β -glucosidase enzyme, common to all *Listeria* spp. and to a few strains of Enterococci and Bacilli.

The specific differential activity of this agar is obtained with a proprietary lecithin substrate for the detection of the phospholipase enzyme present in the *L. monocytogenes* colonies growing on this media. This enzyme activity will result in a halo of precipitation surrounding the target colonies.

With the combination of both the chromogenic and phospholipase enzyme reactions, it is possible to differentiate *Listeria monocytogenes* (blue colonies surrounded by an opaque halo) from other *Listeria* spp (blue colonies without an opaque halo).

Typical Formulation

Meat Peptone	18.0 g/L
Tryptone	6.0 g/L
Yeast Extract	10.0 g/L
Lithium Chloride	10.0 g/L
Sodium Chloride	5.0 g/L
Disodium Hydrogen Orthophosphate Anhydrous	2.5 g/L
Sodium Pyruvate	2.0 g/L
Glucose	2.0 g/L
Glycerophosphate	1.0 g/L
Magnesium Sulphate	0.5 g/L
5-Bromo-4-Chloro-3-Indolyl- β -d-Glucopyranoside	0.05 g/L
Agar	13.5 g/L

Final pH: 7.2 \pm 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

X072 Polymyxin & Ceftazidime Selective Supplement – 1 vial per 500mL

X010 (or X210) *Listeria* Selective Diagnostic Supplement – 1 vial per 500mL

Precaution

Refer to SDS

Preparation

1. Suspend 70.5 grams of the medium in 950mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.



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Technical Specification Sheet



- Cool to 45-50°C, and add 2 vials of reconstituted X072 supplement. Swirl to mix.
- Add 2 vials of X010 supplement (**pre- heated to 48-50°C**). Mix well with gentle end-over-end mixing.

Test Procedure

- For the detection of *Listeria monocytogenes* and *Listeria* spp – Refer to ISO 11290-1:2017
- For the enumeration of *Listeria monocytogenes* and *Listeria* spp – Refer to ISO 11290-2:2017

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and beige.

Prepared Appearance: Prepared medium is an opaque, cream-yellow gel.

Minimum QC:

Listeria monocytogenes WDCM 00021
Escherichia coli WDCM 00013 (Inhibited)

Results

Organism	Colony size (mm)	Colony shape	Colony Color
<i>Listeria monocytogenes</i>	1-2	Round, regular	Blue to blue-green, surrounded by opaque halo
<i>Listeria</i> spp.	1-2	Round, regular	Blue to blue-green, without opaque halo

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing or appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures

- Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
- Isolates presumptively identified as *Listeria* spp. and *Listeria monocytogenes* must be subjected to further biochemical tests to confirm their identity. Some strains of *Listeria ivanovii* may demonstrate lecithinase activity.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

- ISO 11290-1:2017 Microbiology of the food chain- Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp.- Part 1: Detection method
- ISO 11290-2:2017 Microbiology of the food chain- Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp.- Part 2: Enumeration method



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