

Listeria monocytogenes isolation protocols

This section described some procedures to extract bacterial DNA from different specimen kind with high efficiency.

The protocols/Kits provided in this section were been tested, by DIATHEVA, on foods derived from meat and cheese (sausage and “mozzarella”cheese) utilizing Listeria monocytogenes PCR detection KIT (code MBK0001).

BACTERIAL CULTURE ENRICHMENT

Homogenate 25 g of sample material in a Stomacher apparatus with 225 ml of an appropriate enrichment medium, such as Listeria Enrichment Broth Base (CM0862) with Listeria Selective Enrichment Supplement (SR0149) modified with 10 mg/l Acriflavine (Oxoid LTD, Basingstoke, England) or Listeria Fraser Broth Base (CM0895) with Fraser Selective Supplement (SR0156) (Oxoid). Incubate for 24 hours at 30°C.

DNA EXTRACTION

Sample volumes for DNA isolation depend on the protocol or kit used.

We recommend the following procedure:

- filtrate through a gauze the enrichment bacterial culture;
- centrifuge 1 ml of the filtrate at 5000g for 10 min at 4°C;
- remove and discard the supernatant;
- bacterial pellets could be stored at -20°C until use or processed immediately;
- four different extraction methods are suggested.

- 1) Bacterial DNA extraction KIT –DIATHEVA (**DIATHEVA – Fano, ITALY**)
- 2) Dnasy Tissue –Qiagen (**QIAGEN GmbH, Hilden, Germany**)
- 3) Puregene Yeast & Gram-positive Bacteria Kit - GENTRA (**Gentra systems, Minneapolis, USA**)
- 4) PHENOL-CHLOROFORM EXTRACTION

1) Bacterial DNA extraction KIT –DIATHEVA (DIATHEVA – Fano, ITALY)

[Performe the extraction according to the manufacturer protocol](#)

2) DNEASY TISSUE KIT - QIAGEN (QIAGEN GmbH, Hilden, Germany)

Performe the extraction according to the manufacturer protocol: "Isolation of genomic DNA from Gram-positive bacteria".

Optional: after incubating at 37°C with enzymatic lysis buffer (see DNeasy tissue handbook), we suggest the addition of 20 µl 10 mg/ml RNase A (not included). After 10 min at room temperature, continue with the kit procedure.

3) PUREGENE Yeast & Gram-positive Bacteria Kit - GENTRA (Gentra systems, Minneapolis, USA)

Process samples as described in the manufacturer extraction protocol with the following clarifications: execute the optional lysis incubation at 80°C for 5 min; performe RNase reaction for at least 40 min; finally hydrate DNA for 1 h at 65°C.

4) PHENOL-CHLOROFORM EXTRACTION

DNA extraction could be performed according to the standard phenol:chloroform extraction method (8). Briefly, resuspend the bacterial pellet with 0.5 ml lysis buffer (8 M Urea, 0.3 M NaCl, 10 mM Tris-HCl pH 7.4) and add 0.5 ml of 10% sodium dodecyl sulfate. Incubate at 37°C for 20 min. Mix the lysate with 2 volumes of phenol:chloroform buffer (8) for 10 min and centrifuge at 4000g for 10 min or at 12000g for 3 min. Transfer the aqueous phase to a fresh tube and discard the organic phase. If necessary repeat the phenol:chloroform step. Add an equal volume of chloroform:isoamyl alcohol (24:1) to the aqueous phase, mix and centrifuge as above. Recover DNA in the aqueous phase adding 2.5 volume of cold ethanol and 1/10 volume of 3.0 M sodium acetate pH 5.2. Incubate at -20°C for at least 30 min and centrifuge at 12000g for 15 min. Wash the pellet with 70% ethanol for 5 min at 12000g and air dry. Resuspend DNA in a suitable volume of double distilled water (e.g. 50-200 µl).

IMPORTANT: For isolation of *L. monocytogenes* DNA from milk samples without any enrichment culturing step, we recommend the use of "[LISTERIA MONOCYTOGENES DNA isolation Kit: Milk](#)" Product Number # MBK0002.