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Fast DNA Extraction kit

MBK0061 50 extractions
MBK0095 100 extractions

INTENDED USE

The kit is formulated for the rapid preparation of genomic DNA from Gram negative bacteria starting from bacterial enrichment culture.

PRODUCT DESCRIPTION

The protocol is based on thermal lysis that permits to obtain, in only 30 minutes, a DNA extracted suitable for Real-Time PCR assay.
The system is able to isolate, after an enrichment step, DNA from a wide range of food matrices such as cheeses, raw meats and meat products, fruits and vegetables, fishes and seafood products, eggs and derivatives. This kit could not be used for the isolation of bacterial DNA from primary production samples (feces, bootswab, etc.).
This kit can be used for food testing for the detection of pathogens such as *Salmonella* and pathogenic *Escherichia coli* in combination with the Real-Time PCR kits shown below:

Pathogens to be detected	Sample enrichment	Real-Time PCR kit
Salmonella spp.*	ISO 6579	Salmonella spp. FLUO kit (MBK0054-MBK0057)
E. coli O157	ISO 16654 ISO 13136 USDA MLG 5B.02	E. coli O157 FLUO kit (MBK0071)
Shiga Toxing producing *E. coli*	ISO 13136 USDA MLG 5B.02	STEC FLUO detection kit (MBK0068) STEC serotypes FLUO kit (MBK0074)

*For *Salmonella* spp. detection methods please refer to Annex 1.

KIT CONTENTS

Component	50 extractions		100 extractions	
	N vials	Volume	N vials	Volume
Buffer A	2	27.5 ml	4	27.5 ml
Buffer B	1	8.5 ml	2	8.5 m

SHIPPING CONDITIONS

Shipping at room temperature has no dedrimental effect on the performance of this kit

STORAGE

The product should be stored at room temperature (15-25°C).

CUSTOMER-SUPPLIED REAGENTS AND EQUIPMENT

- Heating block suitable for 1.5 ml microcentrifuge tubes and capable of attaining a temperature of 100°C. Alternatively, a water bath may be used;
- Vortex apparatus;
- Sterile filter tips and micropipettes;
- 1.5 ml microcentrifuge tubes.

1. PROCEDURE

All centrifugation steps are carried out in a benchtop microcentrifuge. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\ (r)}}$$

1.1 SAMPLE PREPARATION

Enrichment media must be warmed at room temperature before using it. It is strongly recommended the use of stomacher bags with filter.
Prepare the sample placing **x** g of food sample into a stomacher bag with filter and add **y** ml of the enrichment culture medium. The sample must be diluted 1:10 in the enrichment medium (for example 25 g in 225 ml, 10 g in 90 ml). Homogenize the sample and incubate it at temperature and times described in the ISO reference methods.

Note: if the food samples are being prepared for food control reasons, respect the ISO standard guidelines for incubation temperatures and times.

For *Salmonella* spp. detection methods please refer to Annex 1.

1.2 DNA EXTRACTION

Before starting:

- Preheat a heating block or water bath to 100 °C for use in step 7.
- Cool Buffer B to 4-5 °C immediately before use.

1. After the enrichment step, shake the suspension to homogenize the culture and then allow any debris to decant before collecting sample.

2. Transfer up to 1 ml of bacterial culture to a microcentrifuge tube.
It is very important to avoid the collection of food particles. For food samples with a fatty supernatant collect the sample just below this layer.

3. Centrifuge at 10 000 rpm for 4 minutes. Discard the supernatant carefully taking care to do not disrupt the pellet.

4. Add 1 ml of Buffer A and resuspend the pellet by pipetting the reagent up and down in the tube.

5. Centrifuge at 10 000 rpm for 5 minutes. Discard the supernatant carefully taking care to do not disrupt the pellet.

6. Add 150 µl of cold Buffer B, resuspend the pellet by pipetting the reagent up and down in the tube.

Note: for food samples rich of starch, such as flour, may be required more volume (from 150 to 250 µl).

The pellet must be totally resuspended, if it does not happen proceed with step 7.1.

7. Place the microcentrifuge tube into a heating block or a water bath set to 100 °C. Heat/Boil the sample for 15 minutes. Proceed with step 8.

7.1 Place the microcentrifuge tube into a heating block or a water bath set to 100 °C. Heat/Boil the sample for 15 minutes. During this incubation time, vortexing every 5 minutes to resuspend totally the pellet.

8. Centrifuge at 12 000 rpm for 5 minutes, transfer all the supernatant in a new 1.5 ml microcentrifuge tube taking care to do not disrupt the pellet, and if the sample is fatty just below this layer.

9. Homogenize the genomic DNA by gentle vortexing for a few seconds before using for downstream processes.

The genomic DNA can be stored at 2-8 °C for a few days. For longer term storage, -20 °C is recommended. Before reusing it always allows thawing and mixing well by gentle vortexing.

Annex 1

Procedure for the extraction of *Salmonella* spp. DNA from food and environmental samples

PRINCIPLE OF THE METHOD

The sample is inoculated in Buffered Peptone Water and incubated as described in the reference method ISO 6579. After the enrichment step, the DNA is extracted from culture using *Fast DNA Extraction kit* or the *Bacterial DNA Isolation Single Step*. The DNA extracted is amplified in Real-Time PCR with the *Salmonella spp. FLUO kit*. The kit provides an easy-to-use mastermix, enzyme and positive control for the successful amplification and detection of DNA from *Salmonella* spp., using dual-labelled probes. The presence of an Internal Amplification Control allows to monitor the presence of inhibitory factors, ensuring reliability of negative results.

Specifications	**Details**
Target	*Salmonella* spp.
DNA extraction kit	Fast DNA Extraction kit (MBK0061 format 50 extractions)
Real-Time PCR	Salmonella spp. FLUO kit (MBK0054 50 reactions / MBK0057 100 reactions)
Enrichment broth	Buffered Peptone Water
Enrichment temperature and times	37°C±1°C for 18±2 h
Type of samples	Food sample (meat, meat products, fish and seafood products, fruit and vegetables, flours, dairy products, eggs and derivatives etc.) Environmental samples (swab and sponges)

SAMPLE ENRICHMENT

The Buffered Peptone Water must be warmed at room temperature before using it.

It is strongly recommended the use of stomacher bags with filter.

Prepare the sample placing **x** gr of sample into a stomacher bag with filter and add **y** ml of Buffered Peptone Water. The sample must be diluted 1:10 in the enrichment medium (for example 25 gr in 225 ml, 10 gr in 90 ml).

Homogenize the sample and incubate it at 37°C±1°C for 18±2 h.

PROCEDURE

Before starting:

- Preheat a heating block or water bath to 100 °C for use in step 7.
- Cool Buffer B to 4-5 °C immediately before use.

5. After the enrichment step, shake the suspension to homogenize the culture and then allow any debris to decant before collecting sample.

6. Transfer up to 1 ml of bacterial culture to a microcentrifuge tube. It is very important to avoid the collection of food particles. For food samples with a fatty supernatant collect the sample just below this layer.

7. Centrifuge at 10 000 rpm for 4 minutes. Discard the supernatant carefully taking care to do not disrupt the pellet.

8. Add 1 ml of Buffer A and resuspend the pellet by pipetting the reagent up and down in the tube.

5. Centrifuge at 10 000 rpm for 5 minutes. Discard the supernatant carefully taking care to do not disrupt the pellet.

6. Add 150 µl of cold Buffer B, resuspend the pellet by pipetting the reagent up and down in the tube.

Note: for food samples rich of starch, such as flour, may be required more volume (from 150 to 250 µl).

The pellet must be totally resuspended, if it does not happen proceed with step 7.1.

7. Place the microcentrifuge tube into a heating block or a water bath set to 100 °C. Heat/Boil the sample for 15 minutes. Proceed with step 8.

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8. Centrifuge at 12 000 rpm for 5 minutes, transfer all the supernatant in a new 1.5 ml microcentrifuge tube taking care to do not disrupt the pellet, and if the sample is fatty just below this layer.

9. Homogenize the genomic DNA by gentle vortexing for a few seconds before using for downstream processes.

The genomic DNA can be stored at 2-8 °C for a few days. For longer term storage, -20 °C is recommended. Before reusing it always allows thawing and mixing well by gentle vortexing.