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Bacterial DNA Isolation Single Step
MBK0076 4 x 96 well plates

INTENDED USE

Bacterial DNA Isolation Single Step allows the isolation of genomic DNA from both gram negative and grampositive bacteria with one single procedure, starting from bacterial cultures. This kit can be used for food testing for the DNA extraction of pathogens such as *Salmonella, Listeria monocytogenes* 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)
*L. monocytogenes**	ISO 11290	Listeria monocytogenes FLUO kit (MBK0056 RG/PL-MBK0077-PL)
E. coli O157	ISO 16654 ISO 13136 USDA MLG 5B.02	E. coli O157 FLUO kit (MBK0071)
Salmonella spp. and L. monocytogenes	Multipathogen Enrichment medium (MKZ0002)	L. monocytogenes and Salmonella spp. FLUO kit (MBK0053)
Salmonella L. monocytogenes and *E. coli* O157	Multipathogen Enrichment medium (MKZ0002)	Multipathogen FLUO kit (MBK0019)
Shiga Toxing producing *E. coli*	ISO 13136 USDA MLG 5B.02	STEC FLUO detection kit (MBK0068)

*Please refer to Annex 1 and Annex 2 for the complete description of *Salmonella* spp. and *L. monocytogenes* detection methods.

PRODUCT DESCRIPTION

The Bacterial DNA Isolation Single Step is the easiest handling and fastest DNA purification kit containing a single buffer system and a one-step DNA purification after lysis. Proteins, detergents and low molecular weight compounds are retained by the CleanPlates96 while DNA passes through the column during a short, one-step purification procedure. The obtained DNA is suitable for all common enzymatic reactions (restriction digests, Real-Time PCR, PCR, genotyping etc.).
The system is able to isolate DNA from a wide range of food matrices such as cheeses, raw meats and meat products, fruits and vegetables, flours, fishes and seafood products, eggs and derivatives.

KIT CONTENTS

The kit contains all necessary reagents for lysis and subsequent DNA purification.

Component	Volume
Washing Buffer	2 x 125 ml
Buffer B	50 ml
SDS Solution	50 ml
Proteinase K	4.5 ml
Prep Solution	150 ml
RNase A	11 ml
Lysozyme	150 mg (6 ml)
DTT	1.5 ml
EDTA	1.5 ml
CleanPlates96	4
deep-well plates	8
Sealing tapes	12
Alu sealing tapes	12

SHIPPING CONDITIONS

Shipment is carried out at different temperatures: -20°C and Room temperatures. Upon arrival store each component as indicate below.

STORAGE

The components of the kit require different storage temperatures:

- Lysozyme and DTT should be stored at -18°C to -25°C;
- Proteinase K, Buffer B, Prep Solution, EDTA, and RNase A must be stored at +2°C to +8°C;
- SDS Solution, Washing Buffer, CleanPlates96 can be stored at room temperature (+20°C to +25°C).

If properly stored, see expiration date for the stability of the kit.

PRECAUTIONS

The user should always pay attention to:

- Use pipette tips with aerosol preventive filters;
- Open the deep-well plate carefully to avoid any possible contamination;
- Wear a suitable lab coat;
- Change gloves often, especially if you suspect a possible contamination of them;
- After DNA extraction clean works space periodically with at least 10% bleach or another decontaminant agent.

CUSTOMER-SUPPLIED REAGENTS AND EQUIPMENT

- Stomacher® masticator or equivalent for homogenizing test samples;
- Incubator for sample microbiological enrichment;
- Magnetic stir plate;
- Benchtop centrifuge;
- Vacuum Pump and manifold (facultative);
- Thermomixer with deep-well plate block;
- Vortex apparatus;
- Sterile filter tips, single and multichannel pipettes;
- 50 ml Tubes;
- Multichannel pipette reservoir;
- Powder free gloves;
- Solution of 5% bleach.

1. PROCEDURE

All centrifugation steps are carried out in a benchtop centrifuge. Various speeds are required for different steps, so please check your centrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

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

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary g-force.

1.1 SAMPLE PREPARATION

Sample enrichment (if the kit is used for the isolation of bacterial genomic DNA from foods or other samples)

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** gr 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 gr in 225 ml, 10 gr in 90 ml). Homogenize the sample and incubate it according to the recommended temperature and time for the bacteria.

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

For *Salmonella* spp. and *L. monocytogenes* detection methods please refer to Annex 1 and Annex 2.

1.2 DNA EXTRACTION

Before starting:

- Reconstitute the Lysozyme in 6 ml of molecular biology grade water, mix thoroughly by vortexing and aliquot in working fractions, store the fractions at -18 °C to -25 °C;
- Preheat a thermomixer to 56 °C;
- **Equilibrate CleanPlates96** by adding 350 µl of Prep Solution on each well, incubate for at least 5 minutes at room temperature. Centrifuge at 350 x g for 1 minute or apply vacuum for 30 to 60 sec to remove excess buffer. Discard deep-well plate and place the CleanPlates96 into a new deep-well plate. Use equilibrated CleanPlate96 or store closed at +2 °C to +8 °C and use within one week.

Preparation of Lysis Pre-Mixes :

LB1	**Lysis Buffer LB1**	**1 sample**	**1 plate**	**2 plates**	**3 plates**	**4 plates**
	Buffer B	90 µl	9.9 ml	19.8 ml	29.7 ml	39.6 ml
	Lysozyme	10 µl	1.1 ml	2.2 ml	3.3 ml	4.4 ml
	RNase A	20 µl	2.2 ml	4.4 ml	6.6 ml	8.8 ml
Mix by vortexing. Add **120 µl** of Buffer LB1 to each sample. The Lysis Buffer LB1 is stable for **one week** if stored at +2°C to +8°C.						
LB2	**Lysis Buffer LB2**	**1 sample**	**1 plate**	**2 plates**	**3 plates**	**4 plates**
	SDS Solution	90 µl	9.9 ml	19.8 ml	29.7 ml	39.6 ml
	Proteinase K	10 µl	1.1 ml	2.2 ml	3.3 ml	4.4 ml
	DTT	2.5 µl	0.27 ml	0.55 ml	0.82 ml	1.1 ml
	EDTA	2.0 µl	0.22 ml	0.44 ml	0.66 ml	0.88 ml
Mix thoroughly by vortexing. Add **100 µl** of Buffer LB2 of each sample. The Lysis Buffer LB2 is stable for **1 working day** at +2°C to +8°C.						

Protocol

A. Lysis

1. Transfer up to 500 µl of culture to a deep-well plate.

2. Centrifuge at 2000 *x g* for 10 minutes. Discard the supernatant carefully taking care to do not disrupt the pellet.

3. Add 90 µl of Buffer B, 10 µl of Lysozyme and 20 µl of RNase A* to the cells pellet. Resuspend cells gently by pipetting up and down. Close the deep-well plate using an alu sealing tape. Incubate with shaking (56°C, 200 rpm, 20 min) in a thermomixer.

4. Add 90 µl of SDS solution, 10 µl of Proteinase K, 2.5 µl of DTT and 2 µl of EDTA* to each sample. Close the deep-well plate using an alu sealing tape. Incubate with shaking (56°C, 200 rpm, 60 min) in a thermomixer.

*prepare pre-mixes see the page before.

B. Purification of DNA

Transfer 100 µl of the lysate to an **equilibrated** CleanPlates96. Incubate for 3 minutes at room temperature. Centrifuge at 700 x g for 1 min or apply vacuum for 1 min. The eluate contains the purified DNA.
The genomic DNA can be stored at 2°C to 8°C for a few days. For longer term storage -20°C is recommended.

1.3 QUANTIFICATION OF DNA

We recommend to determine the DNA concentration:
-Using the fluorescent dye Picogreen® or similar;
-Comparing the fluorescence intensity of DNA bands of unknown concentration with standards, e.g. in ethidium bromide stained agarose gels.

Please notice:
The use of absorption measurement at 260 nm (A_{260}) in a spectrophotometer (e.g. NanoDrop®) for determination of DNA concentration is system related not recommended. For details and possible workarounds for your specific application please contact the technical service of Diatheva s.r.l.

Annex 1

Procedure for the extraction of *Salmonella* spp. DNA from food, primary production 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	Bacterial DNA Isolation Single Step (MBK0063 format 50 extractions/MBK0076 4 x 96 deepwell) or 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 samples (meat, meat products, fish and seafood products, fruit and vegetables, flours, dairy products, eggs and derivatives etc.) Environmental samples (swab and sponges) Primary production samples (faeces, boot socks, etc.)

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 FOR FOOD AND ENVIRONMENTAL SAMPLE (EXCLUDING PRIMARY PRODUCTION SAMPLE)

Follow the instruction describe above (1.2 of the protocol) for the reconstitution of Lysozyme, preparations of Lysis mixes and equilibration of CleanPlates96.

A. Lysis

1. Transfer up to 500 µl of culture to a deep-well plate. It is very important to avoid the collection of sample particles. For food sample with a fatty supernatant collect the sample just below this layer.

2. Centrifuge at 2000 *x g* for 10 minutes. Discard the supernatant carefully taking care to do not disrupt the pellet.

3. Add 90 µl of Buffer B, 10 µl of Lysozyme and 20 µl of RNase A* to the cells pellet. Resuspend cells gently by pipetting up and down. Close the deep-well plate using an alu sealing tape. Incubate with shaking (56°C, 1000 rpm, 20 min) in a thermomixer.

4. Add 90 µl of SDS solution, 10 µl of Proteinase K, 2.5 µl of DTT and 2 µl of EDTA* to each sample. Close the deep-well plate using an alu sealing tape. Incubate with shaking (56°C, 1000 rpm, 60 min) in a thermomixer.

*prepare pre-mixes.

B. Purification of DNA

Transfer 100 µl of the lysate to an **equilibrated** CleanPlates96. Incubate for 3 minutes at room temperature. Centrifuge at 700 x g for 1 min or apply vacuum for 1 min. The eluate contains the purified DNA.

The genomic DNA can be stored at 2°C to 8°C for a few days. For longer term storage -20°C is recommended.

PROCEDURE FOR PRIMARY PRODUCTION SAMPLE

Follow the instruction describe above (1.2 of the protocol) for the reconstitution of Lysozyme, preparations of Lysis mixes and equilibration of CleanPlates96.

A. Lysis

1. After the enrichment step, transfer 10 ml of culture in a 15-50 ml tube. Centrifuge at 100 x g for 3 minutes.

2. Transfer up to 500 µl of the supernatant to a deep-well plate.

3. Centrifuge at 2000 x g for 10 minutes. Discard the supernatant carefully taking care to do not disrupt the pellet.

4. Add 90 µl of Buffer B, 10 µl of Lysozyme and 20 µl of RNase A* to the cells pellet. Resuspend cells gently by pipetting up and down. Close the deep-well plate using an alu sealing tape. Incubate with shaking (56°C,900-1000 rpm, 20 min) in a thermomixer.

5. Add 90 µl of SDS solution, 10 µl of Proteinase K, 2.5 µl of DTT and 2 µl of EDTA* to each sample. Close the deep-well plate using an alu sealing tape. Incubate with shaking (56°C, 900-1000 rpm, 60 min) in a thermomixer.

* prepare pre-mixes see.

B. Purification of DNA

Transfer 100 µl of the lysate to an **equilibrated** CleanPlates96. Incubate for 3 minutes at room temperature. Centrifuge at 700 x g for 1 min or apply vacuum for 1 min. The eluate contains the purified DNA.
The genomic DNA can be stored at 2°C to 8°C for a few days. For longer term storage -20°C is recommended.

Annex 2

Procedure for the extraction of *L. monocytogenes* DNA from food and environmental samples

INTRODUCTION

The sample is inoculated in Half Fraser broth and incubated as described in the reference method ISO 11290. After the enrichment step, the DNA is extracted from culture using *Bacterial DNA Isolation Single Step*. The DNA extracted is amplified in Real-Time PCR with the *Listeria monocytogenes FLUO kit*.
The kit provides an easy-to-use mastermix, enzyme and positive control for the successful amplification and detection of DNA from *L. monocytogenes*, 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	*L. monocytogenes*
DNA extraction kit	Bacterial DNA Isolation Single Step (MBK0063 format 50 extraction or MBK0076 4 x 96 deepwell)
Real-Time PCR	*Listeria monocytogenes FLUO kit* (MBK0056 PL/RG-50 reactions or MBK0077 PL-384 reactions).
Enrichment broth	Half Fraser broth
Enrichment temperature and times	30±1°C for 25±1 h
Type of samples	Food samples (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 Half Fraser broth 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 Half Fraser. 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 25±1h at 30±1°C in Half Fraser.

PROCEDURE FOR FOOD AND ENVIRONMENTAL SAMPLE

Follow the instruction describe above (1.2 of the protocol) for the reconstitution of Lysozyme, preparations of Lysis mixes and equilibration of CleanPlates96.

A. Lysis

1. Transfer up to 500 µl of decanted culture to a deep-well plate. It is very important to avoid the collection of sample particles. For food samples with a fatty supernatant collect the sample just below this layer.

2. Centrifuge at 2000 x g for 10 minutes. Discard the supernatant carefully taking care to do not disrupt the pellet.

Optional step

3. For fatty samples, it is suggested to proceed with a washing step. Add 600 µl of Washing buffer and resuspend the cells pellet by pipetting up and down. Centrifuge at 2000 x g for 10 minutes and discard the supernatant carefully taking care to do not disrupt the pellet.

4. Add 90 µl of Buffer B, 10 µl of Lysozyme and 20 µl of RNase A* to the cells pellet. Resuspend cells gently by pipetting up and down. Close the deep-well plate using an alu sealing tape. Incubate with shaking (56°C, 900-1000 rpm, 20 min) in a thermomixer.

5. Add 90 µl of SDS solution, 10 µl of Proteinase K, 2.5 µl of DTT and 2 µl of EDTA* to each sample. Close the deep-well plate using an alu sealing tape. Incubate with shaking (56°C, 900-1000 rpm, 60 min) in a thermomixer.

*prepare pre-mixes.

B. Purification of DNA

Transfer 100 µl of the lysate to an **equilibrated** CleanPlates96. Incubate for 3 minutes at room temperature. Centrifuge at 700 x g for 1 min or apply vacuum for 1 min. The eluate contains the purified DNA.