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For research use only

PREP-L

Kit for sample pretreatment with lysozyme while processing of DNA isolation

INSTRUCTION FOR USE

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REF P-019-N/8EU
P-018-N/8EU

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1. INTENDED USE

The **PREP-L** kit is intended for lysozyme pretreatment of human biological material (faeces), as well as bacterial cultures obtained from this biomaterial for further extraction of bacterial DNA for subsequent analysis by polymerase chain reaction.

This medical device is an auxiliary agent for *in vitro* diagnosis.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **PREP-L** kit.

The **PREP-L** kit can be used in research practice.

Potential users: personnel performing the collection and preprocessing of clinical material, as well as specialists qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

The method is based on the enzymatic destruction by lysozyme (muramidase) of peptidoglycans (in particular, murein) that are part of the cell walls of microorganisms (up to 50-80% in the cell walls of Gram-positive bacteria) by two different complementary mechanisms: first, enzymatic hydrolysis of the N-glycoside bonds that bind polysaccharides and structural glycopeptides of the cell wall, leading to its lysis; second, damage to the cytoplasmic membrane by the cationic mechanism, when lysozyme molecules are incorporated into the cell membrane of microorganisms, forming pores in it and causing osmotic cell death, in order to increase the efficiency of nucleic acid extraction during subsequent DNA extraction.

3. CONTENT

The **PREP-L** kit content is represented in Table 1, 2.

Table 1. The **PREP-L** kit content, Set No.1, for P-019-N/8EU

Reagent	Description	Total volume	Amount
Lysozyme	White fine crystalline powder	80 mg (20 mg in each tube)	4 tubes
Buffer for lysozyme dissolution	Colorless transparent liquid	1000 µL (250 µL in each tube)	4 tubes

Table 2. The **PREP-L** kit content, Set No.2, for P-018-N/8EU

Reagent	Description	Total volume	Amount
Lysozyme	White fine crystalline powder	80 mg (20 mg in each tube)	4 tubes
Buffer for lysozyme dissolution	Colorless transparent liquid	1000 µL (250 µL in each tube)	4 tubes
Disposable plastic spatula, non-sterile			36 pcs

All components are ready to use and do not require additional preparation for operation.
The kit is intended for single use and designed for DNA extraction from 32 analyzed samples (including negative controls).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile containers to collect clinical material;

4.2. NA extraction

- Biological safety cabinet class II-III;
- Refrigerator;
- Freezing chamber;
- Vortex mixer;
- High speed centrifuge (RCF(g) at least 14000) for 1.5 mL tubes;
- Solid-state thermostat (temperature range 24-65 °C);
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Pipette stand;
- Tube rack for 1.5 mL tubes;
- RNase and DNase free 1.5 mL microcentrifuge tubes;
- Physiological saline solution 0.9% NaCl (sterile);
- Glycerol (if needed);
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA PLUS** (REF P-002/2EU), **PREP-MB MAX** (REF P-103-N/4EU) extraction kits are recommended);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of the **PREP-L** kit Set No.1 must be stored at temperatures from 2 °C to 8 °C during the storage period.

All components of the **PREP-L** kit Set No.2, except disposable plastic spatula, must be stored at temperatures from 2 °C to 8 °C during the storage period.

Disposable plastic spatula must be stored at temperatures from 2 °C to 25 °C during the storage period.

The kit must be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

Transportation of the **PREP-L** kit is allowed in thermoboxes with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but for no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

Shelf-life of the kit following the first opening of the primary container:

- components of the **PREP-L** kit should be stored at temperatures from 2 °C to 8 °C during the storage period;
- disposable plastic spatula should be stored at temperatures from 2 °C to 25 °C during the storage period.

The kit stored under undue regime should not be used.

An expired the **PREP-L** kit should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **PREP-L** kit to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the

amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

Human faeces (including meconium) and bacterial cultures extracted from faeces are used for the assay.

General requirements

- In order to obtain correct results, the quality of the biological material sample taken for the assay, its storage, transport and pre-treatment are of great importance.
- Inappropriate sampling may result in doubtful results and sample collection may need to be repeated.
- Use RNase- and DNase-free filter tips during biomaterial preparation.
- To prevent contamination, always open the cap of the tube you are working with (sample/reagent introduction, removal of supernatant) and close it afterwards. It is not allowed to work with several tubes with opened caps at the same time.

Sample collection

Faeces (meconium)

Samples of faeces or meconium with mass (volume) 1.0-3.0 g (1.0-3.0 mL) are transferred to a sterile dry flask by a single-use filtered pipette tip or single-use shovel.

Close the flask tightly and mark it.

ATTENTION! Before DNA extraction pre-processing of biological material samples is needed.

Bacterial cultures

Material is taken from liquid and solid media using a disposable microbiological loop or spatula.

Place a single cell colony or 100 µL of liquid medium into a 1.5 mL single use tube containing 500 µL of sterile physiological saline solution.

Close the tube tightly and mark it.

Transportation and storage of the samples

Samples of faeces or meconium may be transported and stored:

- at room temperature (18 °C to 25 °C) – for no more than 6 hours;
- at temperature from 2 °C to 8 °C – for no more than 3 days.

Fecal suspension with glycerin may be transported and stored:

- at minus 20 °C – for 1 week;
- at minus 70 °C – for a prolonged period.

Bacterial cultures may be transported and stored:

- at temperature from 2 °C to 8 °C – for no more than one day;
- at temperature from minus 18 °C to minus 20 °C – for no more than one week;
- at minus 70 °C – for a prolonged period.

ATTENTION! Avoid repeated freezing and thawing of samples.

Sample preparation

Faeces (meconium) - preparation of the suspension

1. Put approximately 0.1-0.2 g (mL) of faeces into the 1.5 mL tube with 1.0 mL of sterile physiological saline solution.
2. Resuspend the contents of the tube thoroughly for 5-10 seconds on a vortex mixer.

If it is impossible to examine the material within 24 hours and/or if long-term storage is necessary, glycerol at a final concentration of 10-15% is added to the fecal suspension in sterile isotonic sodium chloride solution. Samples prepared in this way are frozen only after thorough homogenization and exposure to glycerol for 30-40 minutes.

Bacterial cultures - preparation of the suspension

Resuspend the contents of the tube thoroughly for 5-10 seconds on a vortex mixer.

8. PROCEDURE

General requirements

Use RNase- and DNase-free disposable filter tips during the biomaterial preparation and pretreatment steps.

Tubes containing test samples and negative control sample (C-) must be processed according to the same scheme simultaneously.

Assay procedure

8.1 Preparation of the lysozyme solution

8.1.1. Add 250 µL of buffer for lysozyme dissolution to the tube containing the lysozyme powder. Close the tube cap.

8.1.2. Resuspend for 3-5 seconds on a vortex mixer until the crystals are completely dissolved.

8.1.3. Spin down the drops from the cap for 1-3 seconds on a vortex mixer.

The obtained volume of lysozyme solution is enough to treat eight test samples.

Unused residue of prepared lysozyme solution can be stored at temperatures from minus 18 °C to minus 22 °C for no more than one week.

ATTENTION! Avoid repeated freezing and thawing of solution.

8.2 Sample processing

8.2.1. Faeces

8.2.1.1 Centrifuge tubes with fecal suspension samples at RCF(g) 13000 for 30 seconds at room temperature (18 °C to 25 °C) to settle the debris at the bottom of the tube.

8.2.1.2 Mark one 1.5 mL tube for each test sample and negative control “C-”.

ATTENTION! To prevent contamination, only open the cap of the tube you are working with (sample/reagent adding) and close it afterwards. It is not allowed to work with several tubes with opened caps at the same time.

8.2.1.3 Add 100 µL of the middle fraction from the fecal suspension tubes to each corresponding tube. No samples should be added to the “C-” tube.

8.2.1.4 Add 100 µL of sterile physiological saline solution or the negative control included in the kit for nucleic acid extraction to the “C-” tube in the volume specified in the instruction of the corresponding kit.

8.2.1.5 Add 20 µL of lysozyme solution (see 8.1) to the marked sample tubes and to the “C-” tube. Use separate tip for each sample.

8.2.1.6 Shake the tubes for 3-5 seconds on a vortex mixer.

8.2.1.7 Spin the tubes for 1-3 seconds in a vortex mixer.

8.2.1.8 Incubate the tubes for 60 minutes at room temperature (18 °C to 25 °C) or for 30 minutes at 37 °C. During incubation shake the tubes gently 2-3 times for 3-5 seconds on a vortex mixer.

8.2.1.9 Spin the tubes for 60 seconds in a vortex mixer.

Samples obtained are ready for DNA extraction.

8.2.2. Bacterial cultures

8.2.2.1 Mark one 1.5 mL tube for each test sample and negative control “C-”.

8.2.2.2 Add 100 µL of the bacterial suspension to each corresponding tube. No samples should be added to the “C-” tube.

8.2.2.3 Add 100 µL of sterile physiological saline solution or the negative control included in the kit for nucleic acid extraction to the “C-” tube in the volume specified in the instruction of the corresponding kit.

8.2.2.4 Add 20 µL of lysozyme solution (see 8.1) to the marked sample tubes and to the “C-” tube. Use separate tip for each sample.

8.2.2.5 Shake the tubes for 3-5 seconds on a vortex mixer.

8.2.2.6 Spin the tubes for 1-3 seconds in a vortex mixer.

8.2.2.7 Incubate the tubes for 60 minutes at room temperature (18 °C to 25 °C) or for 30 minutes at 37 °C. During incubation shake the tubes gently 2-3 times for 3-5 seconds on a vortex mixer.

8.2.2.8 Spin the tubes for 60 seconds in a vortex mixer.

Samples obtained are ready for DNA extraction.

DNA extraction

For DNA extraction it is recommended to use a **PREP-NA PLUS** and **PREP-MB MAX** extraction kits manufactured by “DNA-Technology”.

Carry out DNA extraction from the samples pretreated with lysozyme using all the obtained volume of 120 µL according to the instruction to the kit used.

9. SPECIFICATIONS

a. Amount of biomaterial for pretreatment:

100 µL of fecal suspension or 100 µL of microbial suspension according to the instructions for the recommended reagent kits for DNA extraction.

b. DNA yield

In clinical-laboratory tests, efficiency (increase in DNA yield when pre-treating biomaterial with the tested reagent kit) was determined.

In the study of 70 samples of biomaterial (faeces, bacterial cultures) the following performance indicators (increase in the yield of DNA) were obtained:

	Median	Procentile 25	Procentile 75
All types of biomaterial (70 samples, 115 PCR results)			
Efficiency of biomaterial pretreatment with lysozyme at 37 °C for 30 minutes, %	531.0	401.2	1895.3
Efficiency of biomaterial pretreatment with lysozyme at room temperature (18-25 °C) for 60 minutes, %	694.3	401.2	1895.3
Bacterial cultures (30 samples, 60 PCR results)			
Efficiency of biomaterial pretreatment with lysozyme at 37 C for 30 minutes, %	1484.9	900.0	2411.9
Efficiency of biomaterial pretreatment with lysozyme at room temperature (18-25 C) for 60 minutes, %	1895.3	900.0	2411.9
Faeces (40 samples, 55 PCR results)			
Efficiency of biomaterial pretreatment with lysozyme at 37 °C for 30 minutes, %	401.2	401.2	531.0
Efficiency of biomaterial pretreatment with lysozyme at room temperature (18-25 °C) for 60 minutes, %	401.2	298.1	531.0

NOTE - **BacResista GLA REAL-TIME PCR Detection Kit** was used to evaluate efficacy (increase in DNA yield) in clinical-laboratory tests.

c. Results precision

No differences in efficiency were found between the two pretreatment modes (at 37 °C for 30 minutes and at room temperature (18-25 °C) for 60 minutes) for all the claimed biomaterial types:

Comparison of two pretreatment modes, %	Median	Procentile 25	Procentile 75
All types of biomaterial	0.0	-129.8	129.8
Bacterial cultures	0.0	-163.4	326.0
Faeces	0.0	-129.8	103.1

No differences in efficiency were found for the different reagent kit configurations:

	Median	Procentile 25		Procentile 75
Comparison of two sets, %	0.0	-167.7		51.5

ATTENTION! The claimed specifications are guaranteed when DNA extraction is performed with **PREP-NA PLUS (** REF P-002/2EU) and **PREP-MB MAX** (REF P-103-N/4EU) extraction kits.

10. QUALITY CONTROL

"DNA-Technology Research&Production", LLC declares that the abovementioned products meet the provision of the Council Directive 98/79/EC for *in vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service with quality issues of the **PREP-L** kit.

Technical support:

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11. KEY TO SYMBOLS

	Temperature limit		Date of manufacture
	Contains sufficient for <n> tests		Consult instructions for use
	Use-by date		Catalogue number
	Batch code		Manufacturer
	Version		Caution
	Non-sterile		

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