DNA-TECHNOLOGY

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RUO

For research use only

PREP-CM DNA Extraction Kit

INSTRUCTION FOR USE

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REF P-014-N/2ER

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1. INTENDED USE

PREP-CM DNA Extraction Kit is intended for bacterial and fungal DNA extraction from microorganism cultures (including blood cultures) obtained from human biological material for subsequent PCR analysis.

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

The kit is intended for use together with **BacT/ALERT FA Plus** culture medium (bioMérieux, France). The application of the kits does not depend on population and demographic aspects. There are no contradictions for use of the **PREP-CM DNA Extraction Kit.**

The **PREP-CM DNA Extraction Kit** can be used in research practice.

Potential users: personnel qualified in molecular research methods.

Apply the kit only as directed in this instruction for use.

2. METHOD

Method: alkaline cell lysis in the course of thermal incubation.

The efficiency of such DNA extraction from biological material is close to maximum due to high-temperature processing of samples in lysis solution and minimization of losses.

3. CONTENT

The detailed description of content is represented in Table 1.

Table 1. The **PREP-CM DNA Extraction Kit** content, package N, for P-014-N/2ER

Reagent	Description	Total volume	Amount
Lysis solution	Colorless transparent liquid	20 mL	1 vial
Neutralizing solution	Colorless transparent liquid	400 µL	1 tube
Wash solution B	Colorless transparent liquid	42 mL in each	3 vials
Negative control	Colorless transparent liquid	12.5 mL	1 vial

All components are ready to use and do not require additional preparation for operation.

The **PREP-CM DNA Extraction Kit** is designed for DNA extraction from 50 test samples (including negative controls).

4. ADDITIONAL REAGENTS AND EQUIPMENT REQUIRED

4.1. Specimen collection

- BacT/ALERT FA Plus vials (bioMérieux, France),

4.2. DNA extraction

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) at least 16,000) for 1.5 mL tubes;
- Solid-state thermostat with clamp cover maintaining the 90 °C temperature) (DTtherm by DNA-Technology is recommended);
- RNase and DNase free 1.5 mL snap-cap tubes (for example, Eppendorf Safe-Lock Tubes);
- Tube rack for 1.5 mL tubes;

- Single channel pipettes (dispensers covering 2.0-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL; 200 µL; 1,000 µL);
- Pipette stand;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free non-filtered pipette tips for aspirator with trap flask;
- Disposable syringe with 2.0-5.0 mL needle;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of manufacture.

5.1. Storage conditions

All components of the **PREP-CM DNA Extraction Kit** must be stored at temperatures from 2°C to 8°C over the storage period.

WARNING! The excessive temperature can be detrimental to product performance.

5.2. Transport conditions

Transportation of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the kit components.

It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes from 2°C to 25°C for no longer than 5 days.

WARNING! Reagent kits transported with violation of temperature conditions must not be used.

5.3. Shelf-life of the kit following the first opening of the primary container

All components of the kit must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8 °C and out of light over the storage period.

WARNING! Kits stored with violation of storage conditions must not be used

An expired **PREP-CM DNA Extraction Kit** must not be used.

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

The manufacturer guarantees the conformity of **PREP-CM DNA Extraction Kit** to the technical documentation if the storage, transportation and handling requirements are fulfilled.

6. WARNINGS AND PRECAUTIONS

- Molecular biology procedures, such as nucleic acid extraction, PCR amplification and detection require qualified staff to avoid the risk of erroneous or unreliable results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.
- Wear powder-free single-use surgical gloves. Wear work clothes and personal protective equipment while working with pathogenic microorganisms. The work clothes and personal protective equipment must be suitable for work to be performed and comply with health and safety requirements.
- Avoid any direct contact with the biological samples, reagents and materials used to carry out the procedure. Avoid producing spills or generating aerosols. Do not eat/drink components of the kit. Do not inhale gas/fumes/vapor/aerosols produced by the components of the kit. Avoid contact with eyes.
- 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 only be used for one purpose. The pipettes must be of positive displacement type or be used with aerosol barrier pipette tips. The tips employed must be sterile, free from DNases and RNases and 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 to be utilized in a single session.
- Handle and dispose of all biological samples, reagents and materials used to carry out the procedure as if infectious[1, 2]. Any material being exposed to biological samples must be treated with disinfecting solution for at least 30 minutes or autoclaved for 1 hour at 121°C before disposal.
- All of the liquid solutions are designed for single use and cannot be used more than once in amplification reactions.
- Only use the reagents provided in the kit and those recommended by the manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits.
- All laboratory equipment and tools, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, gloves, etc., as well as reagents must 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 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. Never introduce amplification products in the area designed for extraction/preparation of amplification reactions.
- Do not open the tubes after amplification. Avoid producing accidental spills of the amplification products. Dispose of all PCR waste materials (tubes, tips etc.) only in a closed form in a specialized sealed container with disinfectant solution. Waste materials must be removed in accordance with laboratory internal procedures, and with national and international standards.

[1] - All oligonucleotide components are produced by artificial synthesis in compliance with internal quality control protocol. They do not contain blood or products of blood processing.

[2] - Positive control is produced using artificial DNA synthesis technology, it does not contain parts of infectious agents.

- Working surfaces, as well as rooms where NA extraction and PCR are performed, must be disinfected with bactericidal irradiators (UVGI) for 30 minutes before and after the procedure. All surfaces in the laboratory (test tube racks, equipment, tools, etc.) must be treated with disinfecting solution daily.

Emergency actions

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

Skin Contact: If any component of this kit comes into contact with the skin and causes discomfort, remove any contaminated clothing. Rinse the affected area with plenty of soap and water. If pain or irritation occurs, seek medical attention.

Ingestion: If any component of this kit is ingested, rinse the mouth with plenty of potable water. If irritation or discomfort occurs, seek medical attention.

Do not use the kit:
- If the transportation and storage conditions have been violated;
- If the appearance of the reagents does not correspond to the product documentation;
- If the packaging of the kit components is breached;
- After the expiry date.

Adverse health effects are **NOT** anticipated from routine use of this kit in compliance with the current instruction for use.

7. SAMPLES

Microbial cultures grown in **BacT/ALERT FA Plus** vials (bioMérieux, France) with medium and adsorbent for aerobic and facultative anaerobic microorganism extraction from blood and sterile adult and children biological liquids are used for the test. Microorganism growth induces the carbon dioxide emission which makes the sensor at the bottom of the vial change its color. The **BacT/ALERT®** 3D analyzers mark the color changes every ten minutes and analyze them. Multiparameter algorithms, including the unique “threshold” algorithm, allows to register the microbial growth on the earliest stages.

General recommendations

1. PCR test is a direct method of laboratory test, and biological material sampling must be carried out from the site in the body where infectious process is localized. The decision to perform the test should be taken by a consulting physician based on anamnesis and the aspect of disease.
2. The quality of biomaterial sampling, transport and storage conditions, and preliminary treatment are important to comply with in order to receive a correct result.
3. Incorrect sampling may affect the results, in which case repeating of sampling must be carried out.
4. During biomaterial preparation stage use RNase and DNase free filtered pipette tips.
5. Add the solution to the tube containing biomaterial carefully and without touching the walls of the tube. If touching occurs, change the tip.
6. Tip should be changed after each removal of solution from the sample.
7. To avoid contamination only open the cap of the tube that is in work (adding the sample/reagent, supernatant removal) and close it immediately afterwards. It is not allowed to work with several tubes with open caps.

Interfering substances

The presence of interfering substances in samples of biological material can affect sample preparation, reducing the quality and quantity of isolated DNA; be the cause of doubtful (unreliable) and/or false-negative results; and inhibit PCR. A sign of PCR inhibition is the simultaneous absence of amplification of IC and specific product.

BacT/ALERT FA Plus vials (bioMérieux, France) with medium and adsorbent for aerobic and facultative anaerobic microorganism extraction from blood and sterile adult and children biological liquids belong to the generation of mediums with intensive antibiotics neutralization **FAN® Plus**, which improves extraction rate and the speed of detection. FAN® Plus vials provide neutralizing antibiotics with new adsorbent polymer pellets, which can improve the quality of Gram staining. However, the medium itself can serve as a powerful PCR inhibitor. For this reason, additional washes were introduced into the sample preparation algorithm to free the interfering medium and perform a high-quality and efficient DNA extraction for further use for PCR.

Sample collection

Biomaterial collection into **BacT/ALERT** vials, loading into the device and cultivation are carried out according to the manufacturer's instruction.

Transport and storage of the samples

It is allowed to transport and store vials:

- at a temperature from 20°C to 25°C – for no longer than 24 hours;
- at a temperature from 2°C to 8°C – for no longer than 30 days;
- at a temperature from minus 20°C to minus 18°C – for no longer than 90 days.

WARNING! It is only allowed to freeze and thaw samples once.

8. PROCEDURE

General requirements

1. Use DNAse and RNAse filter tips during sample preparation and DNA extraction.
2. Remove supernatant with separate tip for each test tube.
3. Do not touch the tubes walls while adding reagents to the tube containing biological material. If touching occurs, change the tip.
4. To avoid contamination only open the cap of the tube you are working with (adding the sample/reagent, supernatant removal), then close the tube. It is not allowed to work with several tubes with open caps simultaneously.
5. Treat the tubes with samples and negative control ("C-") equally and simultaneously.

DNA extraction

8.1 Prepare and mark the necessary amount of plastic 1.5 mL tubes for the tested cultures and "C-" and pour 1,100 µL of wash solution B into each one.

8.2 Carefully, without touching the precipitate, draw at least 100 µL of culture form the **BacT/ALERT** vial with the grown microorganism culture using sterile disposable syringe with a needle by puncturing the rubber cap after treating it with disinfectant. For each vial use a new syringe.

8.3 Using the syringe, introduce 50–100 µL of culture into the corresponding test tubes. Do not add culture to the "C-" tube.

8.4 Add negative control to the "C-" tube at the volume corresponding to the volume of culture.

8.5 Mix the content of the tubes thoroughly on vortex for 10–20 seconds.

8.6 Centrifuge the tubes at RCF(g) 16,000 for 10 minutes.

8.7 Remove supernatant, leaving 20–30 µL in the tube (precipitate + liquid fraction).

WARNING! Precipitate can stick off the wall of the tube and may not be visualized. Precipitate does not form in the "C-" tube.

8.8 Add 1,400 µL of wash solution B into each tube.

8.9 Mix the content of the tubes thoroughly on vortex for 10–20 seconds.

8.10 Centrifuge the tubes at RCF(g) 16,000 for 5 minutes.

8.11 Remove supernatant, leaving 10–30 µL in the tube (precipitate + liquid fraction).

8.12 Add 400 µL of lysis solution into each tube.

8.13 Mix the content of the tubes thoroughly on vortex for 10–30 seconds.

8.14 Spin down the drops from tube caps on vortex for 10–30 seconds.

8.15 Incubate the tubes at 90 °C for 20 minutes.

WARNING! Tube caps may open during heating. It is recommended to use test tubes with snap-cap (e. g. Eppendorf Safe-Lock Tubes) or programmable solid-state thermostats (e. g. DTtherm manufactured by "DNA Technology").

For thermostat, it is recommended to use the program with active final cooling of tubes, or withdraw the tubes carefully from the thermostate and allow them to cool down to room temperature (18 °C–25 °C) in the upright position.

8.16 Spin down the drops from tube caps on vortex for 10–30 seconds.

8.17 Shake the tube with neutralizing solution on vortex for 1–3 seconds. Centrifuge on vortex for 1–3 seconds to spin down the drops.

8.18 Add 8.0 µL of neutralizing solution into each tube.

8.19 Mix the content of the tubes thoroughly on vortex for 10 seconds.

8.20 Centrifuge the tubes at RCF(g) 16,000 for 1 minute. DNA preparation is ready for use.

The obtained DNA preparations can be stored at 2°C to 8°C for no longer than 1 month or at minus 18°C or lower for no longer than 6 months.

9. SPECIFICATIONS

a. Recommended amount of biomaterial from which DNA preparation can be obtained: from 50 to 100 µL.

The amount of obtained DNA preparation: from 400 µL.

b. The **PREP-CM** kit is compatible with the kits designed for the bacterial and fungal DNA PCR analysis:

- the kit for real-time PCR detection of bacterial genes of resistance to glycopeptide and beta-lactam antibiotics (**BacResista GLA**, **BacResista GLA Van/Mec**);
- Streptococcus agalactiae DNA real-time PCR detection kit (**Streptococcus agalactiae**);
- real-time PCR kit for detection and typing of pathogens causing mycoses from genus Candida, Malassezia, Saccharomyces and Debaryomyces (**MycosoScreen**), manufactured by "DNA-Technology", LLC.

c. Effectiveness characteristics

Type of biomaterial	**Number of tested samples**	**Reagent kit for amplification (abbreviated name)**	**PREP-CM kit efficiency**
Fungi cultures	30	MycosoScreen	100 % (Ptrue = 92.61)
Bacteria cultures	30	Streptococcus agalactiae	100 % (Ptrue = 92.61)
	30	BacResista GLA	100 % (Ptrue = 92.61)
PREP-CM kit total efficiency	90	**100 % (Ptrue = 97.47)**	

d. Within-batch and between-batch precision

Within-batch precision – 100 % (83.16-100).

Between-batch precision - 100 % (83.16-100).

10. QUALITY CONTROL

The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016.

Contact our customer service with quality issues of **PREP-CM DNA Extraction Kit.**

Technical support:

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11. KEY SYMBOLS

RUO	For research use only		Date of manufacture
	Temperature limit		Consult instruction for use
	Contains sufficient for<n>tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Caution