



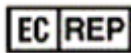
For professional use only

PREP-GS DNA Extraction Kit

PREP-GS PLUS DNA Extraction Kit

PREP-GS Genetics DNA Extraction Kit

INSTRUCTION FOR USE



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P-003/1EU,

P-003/2EU,

P-023/4EU



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1. INTENDED USE

The **PREP-GS DNA Extraction Kit** and **PREP-GS PLUS DNA Extraction Kit** are intended for DNA extraction from biological materials (see Table 1) for further analysis by polymerase chain reaction (PCR). In the **PREP-GS PLUS DNA Extraction Kit** the total volume of purified DNA is larger (300 µL) comparing to standard **PREP-GS DNA Extraction Kit** (100 µL) for more PCR tests. The **PREP-GS Genetics DNA Extraction Kit** is intended for DNA extraction from whole peripheral blood for further DNA genetic testing by PCR.

Table 1. Biological material for DNA/RNA extraction by **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit**

Extraction Kit	PREP-GS DNA Extraction Kit	PREP-GS PLUS DNA Extraction Kit	PREP-GS Genetics DNA Extraction Kit
Biological material	Phlegm, saliva, urine, ejaculate, prostate fluid, cerebrospinal fluid, milk serum, minced tissue, epithelial scrapes from posterior pharyngeal wall, urethra, cervical canal, posterior vaginal vault, biological samples with PCR inhibitors		Peripheral blood

This medical device is an auxiliary agent in clinical laboratory diagnostics.

The application of the kits does not depend on population and demographic aspects. There are no contradictions for use of the **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit**.

The **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

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

2. METHOD

The **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit** are based on nucleic acids release under the action of a chaotropic agent, followed by precipitation and purification of nucleic acids from impurities.

3. CONTENT

The detailed description of content is represented in Tables 2-4.

Table 2. The **PREP-GS DNA Extraction Kit** content, for P-003/1EU

Reagent	Description	Total volume	Amount
Lysis buffer	Light blue slightly foaming liquid	15 mL	1 vial
Sorbent	Brown suspension	2.0 mL (1.0 mL in each tube)	2 tubes
Washout solution №1	Colorless transparent liquid	20 mL	1 vial
Washout solution №2	Colorless transparent liquid	20 mL	1 vial
Washout solution №3	Colorless transparent liquid	20 mL	1 vial
Elution buffer	Colorless transparent liquid	10 mL	1 vial

Table 3. The **PREP-GS PLUS DNA Extraction Kit** content, for P-003/2EU

Reagent	Description	Total volume	Amount
Lysis buffer	Light blue slightly foaming liquid	7.5 mL	1 vial
Sorbent	Brown suspension	1.0 mL	1 tube
Washout solution №1	Colorless transparent liquid	10 mL	1 vial
Washout solution №2	Colorless transparent liquid	10 mL	1 vial
Washout solution №3	Colorless transparent liquid	10 mL	1 vial
Elution buffer	Colorless transparent liquid	15 mL	1 vial

Table 4. The **PREP-GS Genetics DNA Extraction Kit** content, for P-023/4EU

Reagent	Description	Total volume	Amount
Lysis buffer	Light blue slightly foaming liquid	7.2 mL	1 vial
Sorbent	Brown suspension	960 µL	1 tube
Washout solution №1	Colorless transparent liquid	19.2 mL	1 vial
Washout solution №2	Colorless transparent liquid	9.6 mL	1 vial
Washout solution №3	Colorless transparent liquid	9.6 mL	1 vial
Elution buffer	Colorless transparent liquid	14.4 mL	1 vial

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

The kits are intended for single use and designed for 100 analyzed samples (including negative controls) for **PREP-GS DNA Extraction Kit**, 50 analyzed samples (including negative controls) for **PREP-GS PLUS DNA Extraction Kit** and 48 analyzed samples (including negative controls) for **PREP-GS Genetics DNA Extraction Kit**.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs and sterile containers to collect clinical material;
- Sterile tubes containing transport media: “DNA-Technology” made **STOR-M** (**REF** P-910-1/1EU) or **STOR-F** (**REF** P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or physiological saline solution or sterile PBS for the transportation of the sample;
- For blood collection: 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of EDTA at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

4.2. DNA extraction

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF 16000 x g);
- Solid-state thermostat (temperature range 65-98 °C);

- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- RNase and DNase free non-filtered pipette tips for aspirator with trap flask;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

When extracting DNA from phlegm (method 1):

- 10% trisodium phosphate x 12H₂O;
- 1.0M HCl solution;
- 5.0% chloramines solution;
- distilled water.

When extracting DNA from phlegm (method 2):

- mucolysin.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit** must be stored at temperatures from 2 °C to 8 °C over the storage period. The excessive temperature can be detrimental to product performance.

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

It is allowed to transport the kit in thermobox with ice packs by all types of roofed transport at temperatures from 2 °C to 8 °C inside the thermobox.

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

- sorbent, washout solution №2, washout solution №3 and elution buffer should be stored at temperatures from 2 °C to 8 °C during the storage period;
- lysis buffer and washout solution №1 should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period.

The kit stored in under undue regime should not be used.

An expired the **PREP PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction 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-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction 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, 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. 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

The **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit** is designed to extract DNA from a wide variety of biological sample types, such as saliva, phlegm, milk, urine, ejaculate, prostate fluid, cerebrospinal fluid, scrapes of epithelial cells from the posterior pharyngeal wall, urethra, cervical canal, posterior vaginal vault, etc. for **PREP-GS DNA Extraction Kit** and **PREP-GS PLUS DNA Extraction Kits** and peripheral whole blood for **PREP-GS Genetics DNA Extraction Kit**.

Sample collection

Blood sampling

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of EDTA at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant. After taking the material, it is necessary to mix the blood with anticoagulant turning the tube 2 – 3 times.



It is not allowed to use heparin as an anticoagulant.

Phlegm sampling

Sample taking is made in amount no less than 1.0 mL into single-use graduated sterile flasks with wide neck and screwing caps with volume no less than 50 mL.

After sample collection, flask is tightly screwed and marked.

Epithelial scrapes sampling

Procedural limitations for genitourinary smears sampling - local application of medicines, vaginal ultrasound less than 24 hours before the procedure.

Sampling procedure is carried out using special sterile disposable instruments – urogenital swabs, cytobrushes or tampons, depending on the source of clinical material in accordance with established procedures.



In case of pregnancy the use of cytobrushes for genitourinary smears sampling is contraindicated.

The taking of the scrapes is carried out:

- in plastic 1.5 mL tubes with 500 µL of a sterile physiological solution;
- in tubes with transport medium intended by the manufacturer for transportation and storage of samples for PCR.



Remove mucus with sterile cotton swab before taking scrape from cervical channel.

Order of taking:

1. Open the tube.
2. Scrape epithelial cells from the corresponding biotope (posterior pharyngeal wall, urethra, cervical canal, posterior vaginal vault, etc.) with a sterile swab.
3. Put the swab into the tube with transport medium and rinse it thoroughly. Avoid spraying of solution.
4. Remove swab from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the swab.
5. Close the tube tightly and mark it.

Urine sampling

Take the portion (approximately 50 mL) of the first-void urine to sterile container and close it tightly.

Saliva, cerebrospinal fluid, synovial fluid sampling

Collect the saliva, cerebrospinal fluid, synovial fluid (approximately 500 μ L) to the sterile container and close it tightly.

Ejaculate, prostate fluid sampling

Put 100 μ L of the liquid sample into the 1.5 mL tube with transport medium (or alternatively with 500 μ L of sterile buffered saline).

Milk sampling

Collect the sample into the sterile container and close it tightly.

Milk collection period must not exceed 24 hours. Keep at temperatures from 2 °C to 8 °C during the collection period.

Transportation and storage of the samples

Samples may be transported and stored at temperatures from 2 °C to 8 °C for no more than 24 h. When it is impossible to deliver the material in the laboratory during the day, a one-time freezing of the material is allowed. The frozen material is allowed to be stored at temperatures from minus 18 °C to minus 22 °C for one month.

In case of usage transport media, biological material samples are transported and stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Sample preparation

Preparation of the phlegm:

Method 1:

1. Put approximately 500 μ L of biological sample into sterile 1.5 mL tube and close it tightly.
2. Add to the sample an equal volume of 10% triple-substituted sodium phosphate $\times 12\text{H}_2\text{O}$ and mix intensively.
3. Incubate the mixture at 37 °C for 18–24 hours, then neutralize with 1M HCl (down to pH 6.8–7.4).
4. Centrifuge the tube at RCF(g) 100 for 20 minutes.
5. Take out the supernatant into the 5.0% solution of chloramine for disinfection.
6. Add 500 μ L of distilled water to precipitate, mix by pipetting and put to the new 1.5 mL tube.
7. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
8. Remove the supernatant, leaving approximately 100 μ L (precipitate+liquid fraction) in the tube.

Method 2:

1. Add mucolysin to the sampling container in the 5:1 ratio (5 parts of mucolysin to 1 part of phlegm), referring to container calibrations.
2. Close the lid of the container, mix the content and incubate for 20–30 minutes at room temperature, shake the container every 2-3 minutes.

The samples are ready for DNA extraction.

Storage of processed phlegm in a container is accepted at temperatures from 2 °C to 8 °C for one day or at temperatures not above minus 16 °C for along time (in case of repeated DNA extraction necessity).

Preparation of the epithelial scrapes:

1. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
2. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction) in the tube.

The samples are ready for DNA extraction.

Preparation of the urine:

1. Transfer 1.0 mL of the sample to the 1.5 mL tube.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant completely.
4. Add 1.0 mL of sterile buffered saline to the precipitate.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction) in the tube.

The samples are ready for DNA extraction.

Preparation of the saliva, cerebrospinal fluid, synovial fluid:

1. Transfer 500 µL of the sample to the 1.5 mL tube.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction).
4. Add 500 µL of sterile buffered saline to the precipitate.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction).

The samples are ready for DNA extraction.

Preparation of the ejaculate, prostate fluid:

1. Vortex the tubes with samples for 5-10 seconds.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction) in the tube.

The samples are ready for DNA extraction.

Preparation of the milk:

- 1 Mix thoroughly and put 1.0 mL of the sample into the 1.5 mL tube.

The samples are ready for DNA extraction.

8. PROCEDURE

DNA extraction from biological material



Independently of DNA extraction kit used, a negative control sample should go through all stages of DNA extraction. Physiological saline solution can be used as a negative control in volumes as indicated.

Assay procedure:



The lysis buffer and washout solution №1 can form the precipitate. Dissolve it at 50 °C for 15-20 minutes prior to use.

8.1 Mark the required number of 1.5 mL tubes for each test sample and negative control (C-).

Example: to test 5 samples, mark 5 tubes for samples and 1 tube for “C-”. The resulting number of tubes is 6.



For pre-processed samples with obtaining pellet and supernatant (phlegm method 1, saliva, cerebrospinal fluid, urine, ejaculate, prostatic fluid and epithelial scrapes) tubes with 50 µL of material prepared for testing must be marked.

8.2 Prepare the mixture of lysis buffer and sorbent. Add into the one tube:

- 150 x (N+1) µL of lysis buffer,
 - 20 x (N+1) µL of preliminarily resuspended sorbent,
- N is a quantity of the samples to be tested taking to account “C-”.

8.3 Add 170 µL of prepared mixture to marked tubes. Close the tubes.



Always open the tube that you are working with, and close it after handling. It is not allowed to work simultaneously with several tubes with open caps.

8.4 Add 50 µL of prepared sample (**PREP-GS**, **PREP-GS PLUS** kits) or 100 µL of peripheral blood (**PREP-GS Genetics** kit) into the marked tubes. Do not add samples to the “C-” tube and tubes with pre-processed samples with obtaining pellet and supernatant (phlegm method 1, saliva, cerebrospinal fluid, urine, ejaculate, prostatic fluid and epithelial scrapes) (see Table 5).

8.5 Add 50 µL (**PREP-GS**, **PREP-GS PLUS** kits) or 100 µL (**PREP-GS Genetics** kit) of specimen transport medium or sterile buffered saline to “C-” tube (see Table 5).

8.6 Close the tubes tightly and vortex them for 3–5 seconds.

8.7 Incubate the tubes at 50 °C for 20 minutes (**PREP-GS**, **PREP-GS PLUS** kits) or 10 minutes (**PREP-GS Genetics** kit) (see Table 5).

8.8 Centrifuge the tubes at RCF(g) 16000 for 1 minute.

8.9 Remove the supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

8.10 Add 200 µL (**PREP-GS**, **PREP-GS PLUS** kits) or 400 µL (**PREP-GS Genetics** kit) of washout solution №1, close tubes tightly and vortex them for 3–5 seconds (see Table 5).

8.11 Centrifuge the tubes at RCF(g) 16000 for 1 minute.

8.12 Remove the supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

8.13 Add 200 µL of washout solution №2, close tubes tightly and vortex them for 3–5 seconds.

- 8.14** Centrifuge the tubes at RCF(g) 16000 for 1 minute.
- 8.15** Remove the supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.
- 8.16** Add 200 µL of washout solution №3, close tubes tightly and vortex them for 3–5 seconds.
- 8.17** Centrifuge the tubes at RCF(g) 16000 for 1 minute.
- 8.18** Remove the supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.
- 8.19** Open the tubes and dry precipitate by incubation at 50 °C for 5 minutes.
- 8.20** Add to precipitate 100 µL (**PREP-GS** kit) or 300 µL (**PREP-GS PLUS** or **PREP-GS Genetics** kits) of elution buffer, close the tubes tightly and vortex them for 5-10 seconds.
- 8.21** Incubate tubes at 50 °C for 5 minutes (see Table 5).
- 8.22** Centrifuge the tubes at RCF(g) 16000 for 1 minute. Transfer the supernatant into the new tube if sample is to be stored for more than 7 days.

Supernatant containing extracted DNA is ready for adding to PCR-mix.

The obtained DNA sample can be stored at temperatures from 2 °C to 8 °C for no longer than 7 days. Before using the DNA sample for PCR steps, incubate tubes at 50 °C for 5 minutes and then centrifuge the tubes at RCF(g) 16000 for 1 minute. DNA preparation can be stored at temperatures from minus 18 °C to minus 22 °C for no longer than 6 months for **PREP-GS**, **PREP-GS PLUS** kits and no longer than 1 year for **PREP-GS Genetics** kit (see Table 5).

Table 5.

Extraction kit	PREP-GS	PREP-GS PLUS	PREP-GS Genetics
Volume of analyzed sample and negative control sample required for DNA extraction procedure	50 µL		100 µL
Time of incubation in lysis buffer	20 min		10 min
Volume of washout solution №1 required for 1 sample extraction	200 µL		400 µL
Volume of elution buffer required for 1 sample extraction	100 µL	300 µL	
Storage period of purified DNA at temperatures from minus 18 °C to minus 22 °C	up to 6 months		up to 1 year

9. QUALITY CONTROL

"DNA-Technology Research&Production", LLC declares that the above mentioned 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 official representative in EU by quality issues of **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit**.

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10. KEY TO SYMBOLS

	<i>In vitro</i> diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests		Catalogue number
	Use-by date		Manufacturer
	Batch code		Keep away from sunlight
	Caution		Version
	Authorized representative in the European Community		Non-sterile



P-003/1EU,
P-003/2EU,
P-023/4EU



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