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For professional use only

PREP-OPTIMA DNA Extraction Kit

INSTRUCTION FOR USE

EC REP

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REF

P-016-N/2EU
P-016-1/2EU
P-015-N/2EU

VER

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1. INTENDED USE

PREP-OPTIMA DNA Extraction Kit is intended for human, bacterial, viral, and fungal DNA extraction from human biological material (blood; buccal mucosa; smears/scrapings from respiratory, gastrointestinal, and urogenital tracts; urine; faeces; bioptates; amniotic liquid; ejaculate; cerebrospinal fluid; breast milk), as well as for DNA extraction from microbial cultures (bacterial, fungal) received from this biological material for further PCR analysis.

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-OPTIMA DNA Extraction Kit.**

The **PREP-OPTIMA 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

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 reagent kit is available in two versions:

- **PREP-OPTIMA** in manual dosing package (marked as “Package N” and “Package S”).
- **PREP-OPTIMA MAX** in manual dosing package (marked as “Package N”).

The detailed description of content is represented in Tables 1-3.

Table 1. The **PREP-OPTIMA DNA Extraction Kit** content, package N, for P-016-N/2EU

Reagent	Description	Total volume	Amount
Lysis solution	Colorless transparent liquid	20 mL	1 vial
Neutralizing solution	Colorless transparent liquid	400 µL	1 tube

Table 2. **PREP-OPTIMA DNA Extraction Kit** content, package S, for P-016-1/2EU

Reagent	Description	Total volume	Amount
Lysis solution	Colorless transparent liquid	20 mL (400 µL in each)	50 tubes
Neutralizing solution	Colorless transparent liquid	400 µL	1 tube

Table 3. The **PREP-OPTIMA MAX DNA Extraction Kit** content, package N, for P-015-N/2EU

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 CK	Colorless transparent foamy liquid	35 mL	1 vial
Wash solution P	Colorless transparent liquid	70 mL (35 mL in each)	2 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-OPTIMA DNA Extraction Kit** designed for DNA extraction from 50 analyzed samples (including negative controls).

The **PREP-OPTIMA DNA Extraction Kit** (Package S) contains a single use reagent (Lysis solution).

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-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or physiological saline solution (if necessary);
- For blood collection: 2.0 - 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(g) 16000);
- Solid-state thermostat (temperature range 25-90 °C) (for example, TT-1 or TT-2, made by “DNA-Technology”, LLC);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes (RNase and DNase free 1.5 mL snap-cap tubes, for example, Eppendorf Safe-Lock Tubes);
- Physiological saline solution 0.9% NaCl (Sterile) (if necessary);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- 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);
- 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.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

The **PREP-OPTIMA DNA Extraction Kit** must be transported in thermoboxes with ice packs by all types of roofed transport at temperatures inside the thermoboxes corresponding to 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 temperatures from 2 °C to 25 °C inside the thermoboxes but no more than 5 days.

The kit transported with violation of temperature conditions shall not be used.

All components of the **PREP-OPTIMA 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.

Shelf-life of the kit following the first opening of the primary container: the components of the kit must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period.

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

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

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

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

Contact our official representative in EU by quality issues of the **PREP-OPTIMA DNA Extraction Kit.**

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-OPTIMA DNA Extraction Kit** is designed to extract DNA from blood; buccal mucosa; smears/scrapings from respiratory, gastrointestinal, and urogenital tracts; urine; faeces; bioptates; amniotic liquid; ejaculate; cerebrospinal fluid; breast milk, as well as from microbial cultures (bacterial, viral, fungal) received from this biological material.

General recommendations

- PCR study is a direct method of laboratory study, and biological material sampling must be carried out from the site in the body where infectious process is localized. The decision to carry out the study should be taken by a consulting physician based on anamnesis and the aspect of disease.
- The quality of biomaterial sampling, transport and storage conditions, and preliminary treatment are important to comply with in order to receive a correct result.
- In case of sampling from several biotopes repeat the procedure using new swabs and tubes for each biotope.

- Incorrect sampling may affect the results, in which case repeating of sampling must be carried out.
- During of biomaterial preparation stage use RNase and DNase free filtered pipette tips.
- Add the solution to the tube containing biomaterial carefully and without touching the walls of the tube. If touching occurs, change the tip.
- Tip should be changed after each removal of solution from the sample.
- 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. To study the effect of some substances on sample preparation and thus capable of distorting the results of PCR analysis, a simultaneous isolation of DNA from biological material without and with the addition of the tested interferents in concentrations exceeding the potentially possible for these samples was performed.

Evaluation of the effect of interfering substances in samples of biological material on the results of the study are presented in the table below.

Biomaterial type	Interfering substance	Concentration under study
Endogenous substances		
Blood, faeces, urine, smears/scrapes from gastrointestinal tract, amniotic fluid	Bilirubin	171 µmol/L
Blood, faeces, urine, smears/scrapes from gastrointestinal tract, amniotic fluid	Cholesterol	13 µmol/L
Smears/scrapes from gastrointestinal and respiratory tracts, faeces, urine, bioptates, amniotic fluid	Hemoglobin*	1%
Buccal mucosa, bioptates, ejaculate, smears/scrapes from urogenital and respiratory tracts	Mucus (mucin)	5%
Exogenous substances		
Blood	EDTA	up to 2.0 mg/mL
Smears/scrapes from respiratory tract, buccal mucosa	Nasonex	5%
Smears/scrapes from respiratory tract, buccal mucosa	Pinosol	5%
Smears/scrapes from respiratory, gastrointestinal, and urogenital tracts, urine, faeces, bioptates, amniotic fluid, ejaculate, liquor, milk, buccal mucosa	Chlorhexidine bigluconate	5%
Buccal mucosa, smears/scrapes from respiratory, gastrointestinal, and urogenital tracts; urine, milk	Octenisept	5%

* for biomaterial samples isolated using the **PREP-OPTIMA** kit

In order to reduce the number of biological samples with IS, it is necessary to follow the rules of their collection.

Due to the fact that the methodology underlying the **PREP-OPTIMA** sample preparation recommends taking some biological material directly into the lysis solution, it is important to observe their ratio. If the volume of the precipitate is more than one third of the total sample volume in the lysis solution, the efficiency of DNA extraction may decrease.

Each amplification kit has an internal control to assess the quality of DNA extraction and possible PCR inhibition. A sign of complete PCR inhibition is the simultaneous absence of amplification of IC and specific products.

Sample collection

Blood sampling

Peripheral blood sampling is carried out in a vacuum plastic tube. It may be 2.0 - 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of EDTA at a final concentration of 2.0 mg/mL. After taking the material, it is necessary to mix the blood with anticoagulant turning the tube 8 – 10 times.

It is also allowed to use sodium citrate as anticoagulant (if it does not contradict the requirements of the PCR kit used together with the **PREP-OPTIMA MAX** kit).

ATTENION! It is not allowed to use heparin as an anticoagulant.

ATTENION! Intravenous injections of heparin, infusions of parenteral nutrition are not allowed if done less than 6 hours before the test.

Buccal mucosa

ATTENION! Sampling procedure is carried out using a dry swab. It is necessary to keep the solutions away from skin, eyes, and mucous membranes. If the contact occurs, it is necessary to wash the affected area immediately and obtain medical attention.

Sampling procedure is carried out using special sterile disposable instruments – swabs, cytobrushes or tampons in accordance with established procedure.

It is recommended to abstain from eating, smoking, and taking medication perorally two hours prior the biological material sampling. Before sampling rinse mouth with water twice.

Sampling is carried out in 1.5 mL tube with 400 µL of lysis solution.

Order of taking:

1 In order to take the smear, rotate the sterile swab slightly pressing the buccal region.

2 Open the tube.

3 Put the swab into the tube with lysis solution, rotate the swab for 10-15 seconds 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.

Smears/scrapings from respiratory, gastrointestinal, and urogenital tracts

ATTENION! Sampling procedure is carried out using a dry swab. It is necessary to keep the solutions away from skin, eyes, and mucous membranes. If the contact occurs, wash the affected area immediately and obtain medical attention.

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

The taking of the smears\scrapings is carried out in 1.5 mL tube with 400 µL of lysis solution.

Note: If necessary, the material can be collected in 1.5 mL tubes pre-filled with 500 µL of sterile physiological solution or **STOR-F** transport medium.

Note: If necessary, samples may be taken into PreservCyt transport medium for ThinPrep liquid-based cytology (Hologic, USA). Biological material is collected in a vial with transport medium according to the manufacturer's instructions.

Order of taking:

1 Use sterile swab to take a smear/scraping from respiratory, gastrointestinal, and urogenital tracts.

2 Open the tube.

3 Put the swab into the tube with lysis solution or physiological saline solution or transport medium, rotate the swab for 10-15 seconds 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.

Close the tube tightly and mark it.

Urine sampling

Take the portion (approximately 20-30 mL) of the first-void urine to sterile container, close it tightly and mark it.

Faeces sampling

1.0 – 3.0 g (1.0 - 3.0 mL) of faeces should be transported with a separate filtered tip or a single-use spatula to a dry sterile vial.

Close the tube tightly and mark it.

Bioptates sampling (**Sampling is carried out according to an approved procedure)**

Bioptates are transferred to a 1.5 mL tubes with 400 µL lysis solution. After sample collection the tube is tightly closed and marked.

ATTENION! The size of tissue fragment must be no more than 5 mm in length on the longer side or in diameter.

Amniotic liquid sampling (**Sampling is carried out according to an approved procedure)**

Collect 1.0-2.0 mL of amniotic liquid to a dry sterile container.

Close the container tightly and mark it.

Ejaculate sampling (**Sampling is carried out according to an approved procedure)**

Collect ejaculate to a 60 mL dry sterile container.

Close the container tightly and mark it.

Cerebrospinal fluid (**Sampling is carried out according to an approved procedure)**

Collect the cerebrospinal fluid (approximately 500 – 1000 µL) to the 1.5 mL tube.

Close the tube tightly and mark it.

Milk sampling (**Sampling is carried out according to an approved procedure)**

Collect breast milk to a 60 mL dry sterile container.

Close the container tightly and mark it.

Microbial cultures

Sample collection from liquid and solid media is carried out with a single-use inoculation loop or a spatula.

Sample collection is carried out in 1.5 mL tube with 400 µL of lysis solution, no less than 5.0 µL of single cell colony, or no more than 50 µL of liquid medium.

Note: If necessary, the material can be collected in 1.5 mL tubes pre-filled with 500 µL of sterile physiological solution or **STOR-F** transport medium.

Transportation and storage of the samples

Whole blood

It is allowed to transport and store blood samples:

- at a temperature from 2 °C to 8 °C – for no longer than 6 hours from sampling for DNA study of microorganisms in the blood;
- at a temperature from 2 °C to 8 °C – for no longer than 30 days from sampling for human genetic polymorphism study.

ATTENION! It is not allowed to freeze whole blood.

Smears/scrapes from respiratory, gastrointestinal, and urogenital tracts, buccal mucosa, bioptates, microbial cultures

It is allowed to transport and store samples of buccal mucosa, smears/scrapings from respiratory, gastrointestinal, and urogenital tracts, bioptates, and microbial cultures put into **lysis solution**:

- at a temperature from 20 °C to 25 °C – for no longer than 48 hours;
- at a temperature from 2 °C to 8 °C – for no longer than 30 days;
- at a temperature from minus 18 °C to minus 20 °C – for no longer than 90 days.

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

It is allowed to transport and store smears/scrapings from respiratory, gastrointestinal, and urogenital tracts and microbial cultures put into the transport medium for **STOR-F** bioassay or into sterile physiological saline solution:

- at a temperature from 18 °C to 25 °C – for no longer than 48 hours;
- at a temperature from 2 °C to 8 °C – for no longer than 7 days.

In case smears/scrapings from urogenital tract are put into the **transport medium** PreservCyt for cytometry ThinPrep (Hologic, USA), transportation and storage conditions should be determined by the manufacturer.

Urine:

It is allowed to transport and store native and preliminary processed samples of urine:

- at a temperature from 2 °C to 8 °C – for no longer than 24 hours;
- at a temperature from minus 18 °C to minus 20 °C – for no longer than one week;
- at a temperature minus 70 °C – for a prolonged period.

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

Amniotic liquid, ejaculate, cerebrospinal fluid, breast milk

It is allowed to transport and store samples of amniotic liquid, ejaculate, cerebrospinal fluid, breast milk:

- at a temperature from 2 °C to 8 °C – for no longer than 24 hours;
- at a temperature from minus 18 °C to minus 20 °C – for no longer than one week;
- at a temperature minus 70 °C – for a prolonged period.

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

Native faeces samples

It is allowed to transport and store samples of native faeces:

- at room temperature (from 18 °C to 25 °C) – for no longer than 6 hours;

– at a temperature from 2 °C to 8 °C – for no longer than 3 days.

Sample preparation

Smears/scrapings from respiratory, gastrointestinal, and urogenital tracts, microbial cultures taken into physiological saline solution, transport medium STOR-F, transport medium PreservCyt for cytometry ThinPrep (Hologic, USA).

1 Mark an empty tube for negative control sample “C-” and add sterile physiological saline solution or a negative control sample included in the reagent kit in the volume corresponding to the volumes of analyzed samples.

2 Centrifuge the tubes with samples taken into physiological saline solution or transport medium (recommended volume should not exceed 500-600 µL) and the tubes with negative control samples at RCF(g) 16000 for 10 minutes. If the sample is taken into PreservCyt transport medium for cytometry, the volume may be increased to 1000 µL.

3 Remove supernatant, leaving approximately 50 µL or less (precipitation and liquid fraction).

4 Add 400 µL of lysis solution to each tube.

The samples are ready for DNA extraction.

Urine, amniotic liquid, cerebrospinal fluid

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

2 Add 1.0 mL of each sample to the corresponding tubes. The samples are not to be added to the tube “C-”.

3 Add 1.0 mL of sterile physiological saline solution or negative control sample to the tube “C-”.

4 Centrifuge all the tubes at RCF(g) 16000 for 10 minutes.

5 Remove supernatant, leaving approximately 50 µL or less (precipitation and liquid fraction).

6 If the volume of precipitation exceeds 50 µL, add 1.0 mL of sterile physiological saline solution to each tube, mix thoroughly on vortex mixer for 10-30 seconds and centrifuge the tubes at RCF(g) 16000 for 10 minutes.

7 Remove supernatant, leaving approximately 50 µL or less (precipitation and liquid fraction).

8 Add 400 µL of lysis solution to each tube.

The samples are ready for DNA extraction.

Ejaculate

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

2 Add 1.0 mL of sterile physiological saline solution to each tube.

3 Add 100 µL of ejaculate to the corresponding tubes. Ejaculate is not to be added to the tube “C-”.

4 Add 100 µL of sterile physiological saline solution or negative control sample to the tube “C-”.

5 Mix the tubes on vortex mixer for 1-3 seconds.

6 Centrifuge all the tubes at RCF(g) 16000 for 10 minutes.

7 Remove supernatant, leaving approximately 50 µL or less (precipitation and liquid fraction).

8 If the volume of precipitation exceeds 50 µL, add 1.0 mL of sterile physiological saline solution to each tube, mix thoroughly on vortex mixer for 10-30 seconds and centrifuge the tubes at RCF(g) 16000 for 10 minutes.

9 Remove supernatant, leaving approximately 50 µL or less (precipitation and liquid fraction).

10 Add 400 µL of lysis solution to each tube.

The samples are ready for DNA extraction.

Faeces

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

2 Add 1.0 mL of sterile physiological saline solution to each tube.

3 Add faeces samples 5 mm in diameter to the corresponding tubes. Faeces are not to be added to the tube “C-”.

4 Mix the tubes thoroughly on vortex mixer for 10-30 seconds and centrifuge the tubes at RCF(g) 900 for 15 minutes.

5.1. For package S:

- Mark one 1.5 mL tube with 400 µL of lysis solution for each sample and for negative control sample “C-”.
- Transfer 50 µL of supernatant middle fraction into each corresponding tube.

5.2. For package N:

- Mark one empty 1.5 mL tube for each test sample and for negative control “C-“.
- Transfer 50 µl of supernatant middle fraction into each corresponding tube.
- Add 400 µL of lysis solution into each tube.

The samples are ready for DNA extraction.

ATTENTION! This method of DNA extraction is not recommended for large intestine microbiota content study.

Milk

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

2 Add 1.0 mL of each sample to the corresponding tubes. The samples are not to be added to the tube “C-”.

3 Add 1.0 mL of sterile physiological saline solution or negative control sample to the tube “C-”.

4 Mix the tubes on vortex mixer for 1-3 seconds.

5 Centrifuge all the tubes at RCF(g) 16000 for 10 minutes.

6 Remove supernatant, leaving approximately 50 µL or less (precipitation and liquid fraction).

7 Add 400 µL of lysis solution to the supernatant, resuspend it with pipetting without capturing the upper layer of milk fat left on the walls of the tube.

8 Mark one new 1.5 mL tube for each sample.

9 Transport the suspension to the corresponding tubes without capturing the upper layer of milk fat left on the walls of the tube.

10 Add 400 µL of lysis solution to “C-” tube.

The samples are ready for DNA extraction.

8. PROCEDURE

General requirements

1 Use DNAse and RNAse filter tips.

2 It is recommended to 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 which is in work (adding the sample/reagent, supernatant removal), then close the tube. It is not allowed to work with several tubes with open caps simultaneously.

5 Proceed the tubes with samples and negative control ("C-") equally and simultaneously.

DNA extraction

ATTENTION! DNA extraction from blood can only be carried out with the **PREP-OPTIMA MAX** kit. DNA extraction from other biological materials and microbial cultures is carried out with a reagent kit in any version (**PREP-OPTIMA MAX**, **PREP-OPTIMA**).

ATTENTION! It is necessary to prepare negative control sample simultaneously with DNA extraction from biological material and follow all the stages of sample preparation. It is recommended to use lysis solution (Package N, **PREP-OPTIMA**), a new tube with lysis solution (Package S, **PREP-OPTIMA**), or a negative control sample included in the reagent kit (Package N, **PREP-OPTIMA MAX**).

Assay procedure:

8.1 DNA extraction from samples taken into lysis solution (smears/scrapings from respiratory, gastrointestinal, or urogenital tracts, buccal mucosa, bioptates, microbial cultures) and from samples taken after sample preparation

8.1.1 Mix the tubes with samples and negative control sample thoroughly on vortex mixer for 10-30 seconds.

8.1.2 Spin down the drops from caps in vortex mixer for 10-30 seconds. Incubate the tubes at 90 °C for 20 minutes.

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

It is recommended to use active cooling program on thermostat or carefully remove the tubes from thermostat and allow them to cool vertically at room temperature (from 18 °C to 25 °C).

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

DNA preparation is ready to be added to reaction mixture for PCR amplification. The obtained DNA preparation is allowed to be stored at a temperature from minus 20 °C to 8 °C for no longer than 7 days. If the sample is to be used for longer than 7 days:

8.1.4 Transport the corresponding amount of DNA preparation to the new marked 1.5 mL vortex tube.

8.1.5 Shake the tube with neutralizing solution on vortex mixer for 1-3 seconds. Spin down the drops from caps in vortex mixer for 1-3 seconds.

8.1.6 Without touching the edge of the tube, add the sufficient amount of neutralizing solution in ratio 2.0 µL of solution to 100 µL of DNA preparation aliquots (see the table below).

Sample to neutralizing solution ratio:

	Aliquot, µL						
	100	150	200	250	300	350	400
Neutralizing solution, µL	2.0	3.0	4.0	5.0	6.0	7.0	8.0

8.1.7 Mix the tubes thoroughly on vortex mixer for 10 seconds.

The obtained DNA preparation is allowed to be stored at a temperature from 2 °C to 8 °C for no longer than a month, at a temperature from minus 18 °C for no longer than 6 months.

It is necessary to thaw the DNA preparation at room temperature (from 18 °C to 25 °C) or at a temperature from 2 °C to 8 °C, mix the tube on vortex mixer for 1-3 seconds, and centrifuge the tube at RCF(g) 16000 for 1 minute prior to use.

DNA preparation is ready to be added into reaction mixture for PCR amplification.

ATTENTION! It is necessary to comply with regulations of biological sampling. In case of violations in biological sampling, transportation and storage, technological violations in the course of DNA extraction, contamination of tube with inhibiting components, and if the precipitation volume amounts to more than one third of the sample volume in lysis solution, PCR efficiency may decrease to the point of inhibition.

8.2 DNA extraction from samples taking into lysis solution or transport medium with violations at preanalytical stage

8.2.1 Mark one new test tube with 400 µL of lysis solution for each sample and negative control sample “C-”.

8.2.2 Centrifuge the samples with precipitation surpassing one third of the sample volume or with inhibited PCR at RCF(g) 16000 for 30-60 seconds.

8.2.3 Transport 100 µL of supernatant medium fraction to each new corresponding test tube. The sample is not to be added in “C-” tube.

8.2.4 Spin down the drops from caps in vortex mixer for 10-30 seconds. Incubate the tubes at 90 °C for 20 minutes.

ATTENTION! Tube caps may open during warm-up. It is recommended to snap-cap tubes (e. g. Eppendorf Safe-Lock Tubes) or programmable solid-state thermostats (e. g. Gnom Thermostat manufactured by “DNA Technology SPA, LLC”).

It is recommended to use active cooling program on thermostat or carefully remove the tubes from thermostat and allow them to cool vertically at room temperature (from 18 °C to 25 °C).

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

DNA preparation is ready to be added to reaction mixture for PCR amplification. The obtained DNA preparation is allowed to be stored at a temperature from minus 20 °C to 8 °C for no longer than 7 days. If the sample is to be used for longer than 7 days:

8.2.6 Transport the corresponding amount of DNA preparation to the new marked 1.5 mL vortex tube.

8.2.7 Shake the tube with neutralizing solution on vortex mixer for 1-3 seconds. Spin down the drops from caps in vortex mixer for 1-3 seconds.

8.2.8 Without touching the edge of the tube, add the sufficient amount of neutralizing solution in ratio 2.0 µL of solution to 100 µL of DNA preparation aliquots (see the table below).

Sample to neutralizing solution ratio

	Aliquot, µL						
	100	150	200	250	300	350	400
Neutralizing solution, µL	2.0	3.0	4.0	5.0	6.0	7.0	8.0

8.2.9 Mix the tubes thoroughly on vortex mixer for 10 seconds.

The obtained DNA preparation is allowed to be stored at a temperature from 2 °C to 8 °C for no longer than a month, at a temperature from minus 18 °C for no longer than 6 months.

It is necessary to thaw the DNA preparation at room temperature (from 18 °C to 25 °C) or at a temperature from 2 °C to 8 °C, mix the tube on vortex mixer for 1-3 seconds, and centrifuge the tube at RCF(g) 16000 for 1 minute prior to use.

DNA preparation is ready to be added to reaction mixture for PCR amplification.

8.3 DNA extraction from samples taken into lysis solution with violations at preanalytical stage using other DNA extraction kits

If necessary, it is possible to repeat DNA extraction from samples using other reagent kits, e. g. **PREP-GS** or **PREP-NA**.

8.3.1 Mark one new test tube for each sample and negative control sample “C-”.

8.3.2 Centrifuge the samples with inhibited PCR at RCF(g) 16000 for 30-60 seconds.

8.3.3 Transport 50-100 µL of supernatant medium fraction to each new corresponding test tube according to the instruction to the reagent kit.

8.3.4 Add a sufficient amount of negative control sample from the reagent kit or sterile physiological saline solution to the “C-” tube.

8.3.5 Carry out DNA extraction according to the instruction to the reagent kit.

8.4 DNA extraction from blood using PREP-OPTIMA MAX reagent kit only

8.4.1 Mark one new test tube for each sample and negative control sample “C-”.

8.4.2 Add 100 µL of blood for further genetic polymorphism study, or 100-500 µL for microbial DNA extraction to each tube. For microbial DNA extraction, it is recommended to use 500 µL of blood because in case microorganisms are present in the sample increasing the volume of blood for examination will increase the concentration of their DNA in the final preparation. Blood is not to be added in “C-” tube.

8.4.3 Add the negative control sample in the “C-” tube in a volume equal to that of blood in the test tubes.

8.4.4 Add the wash solution CK to all the tubes in the volume equal to that of blood/negative control sample.

8.4.5 Mix the tubes thoroughly on vortex mixer for 10-30 seconds until they are evenly colored. The content of “C-” tube is to remain colorless.

8.4.6 Incubate the tubes vertically at room temperature (from 18 °C to 25 °C) for 5 minutes. During the incubation mix the tubes on vortex mixer 2-3 times for 20-30 seconds each.

8.4.7 Centrifuge the tubes at RCF(g) 16000 for 10 minutes.

8.4.8 Remove supernatant (separate tip for each test tube) without touching the loose clot in the cone part of the tube, leaving approximately 10-20 µL in the tube (precipitation and liquid fraction).

ATTENTION! The clot may not adhere tightly to the wall of the tube.

8.4.9 Add 1400 µL of wash solution P to each test tube.

8.4.10 Mix the tubes thoroughly on vortex mixer for 20-30 seconds trying to break up any precipitation.

8.4.11 Centrifuge the tubes at RCF(g) 16000 for 5 minutes.

8.4.12 Remove supernatant (separate tip for each test tube) without touching the loose clot in the cone part of the tube, leaving approximately 10 µL in the tube (precipitation and liquid fraction).

ATTENTION! The clot may not adhere tightly to the wall of the tube.

8.4.13 Add 100 µL of lysis solution in each tube for microbial DNA extraction, or 300 µL of lysis solution for human DNA extraction for further genetic polymorphisms study.

8.4.14 Mix the tubes thoroughly on vortex mixer for 10-30 seconds trying to break up any precipitation.

8.4.15 Spin down the drops from caps in vortex mixer for 10-30 seconds.

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

ATTENTION! Tube caps may open during warm-up. It is recommended to use snap-cap tubes (e. g. Eppendorf Safe-Lock Tubes) or programmable solid-state thermostats (e. g. Gnom Thermostat manufactured by "DNA Technology SPA, LLC"). It is recommended to use active cooling program on thermostat or carefully remove the tubes from thermostat and allow them to cool vertically at room temperature (from 18 °C to 25 °C).

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

DNA preparation is ready to be added to reaction mixture for PCR amplification. The obtained DNA preparation is allowed to be stored at a temperature from minus 20 °C to 8 °C for no longer than 7 days. If the sample is to be used for longer than 7 days:

8.4.18 Mix the tubes with neutralizing solution on vortex mixer for 1-3 seconds. Spin down the drops from caps in vortex mixer for 1-3 seconds.

8.4.19 Without touching the edge of the tube, add the sufficient amount of neutralizing solution in ratio 2.0 µL of solution to 100 µL of sample or 6.0 µL of solution to 300 µL of sample.

8.4.20 Mix the tubes thoroughly on vortex mixer for 10 seconds.

The obtained DNA preparation is allowed to be stored at a temperature from 2 °C to 8 °C for no longer than a month, at a temperature from minus 18 °C for no longer than 6 months.

It is necessary to thaw the DNA preparation at room temperature (from 18 °C to 25 °C) or at a temperature from 2 °C to 8 °C, mix the tube on vortex mixer for 1-3 seconds, and centrifuge the tube at RCF(g) 16000 for 1 minute prior to use.

DNA preparation is ready to be added to reaction mixture for PCR amplification.

9. SPECIFICATIONS

a. Effectiveness characteristics

Recommended amount of biomaterial from which DNA preparation can be obtained: from 5.0 to 1000 µL [1].

DNA yield (efficiency) is 440-680 ng [2].

Volume of obtained DNA preparation: from 100 to 450 µL.

The effectiveness of the **PREP-OPTIMA** kit was established in clinical trials using additional reagent kits for amplification in the study of samples of biological material.

Type of biomaterial	Number of tested samples	Reagent kit for amplification (abbreviated name)	PREP-OPTIMA kit efficiency
Amniotic fluid	16	SIC*	100 % (Ptrue = 86.6)
Bioptates	12	Streptococcus agalactiae	100 % (Ptrue = 82.54)
Buccal mucosa	30	SIC*	100 % (Ptrue = 92.61)
Blood	36	CMV	100 % (Ptrue = 93.8)
	40	SIC*	100 % (Ptrue = 94.41)
Liquor	16	Streptococcus agalactiae	100 % (Ptrue = 86.6)
Respiratory smears/scrapes	23	Candida albicans	100 % (Ptrue = 90.47)
Gastrointestinal smears/scrapes	27	Candida albicans	100 % (Ptrue = 91.83)
Urogenital smears/scrapes	41	Mycoplasma hominis	100 % (Ptrue = 94.54)
Milk	32	CMV	100 % (Ptrue = 93.06)

[1] functional indicators depend on the type and amount of biomaterial;

[2] DNA yield is given for isolation from 100 µL blood with a leukocyte count ranging from 5.5 x 10^9/L to 7.8 x10^9/L.

Urine	35	CMV	100 % (Ptrue = 93.63)
	22	Streptococcus agalactiae	100 % (Ptrue = 90.06)
Faeces	23	Candida albicans	100 % (Ptrue = 90.47)
Ejaculate	38	Mycoplasma hominis	100 % (Ptrue = 94.12)
Microbial cultures	43	MycosoScreen	100 % (Ptrue = 94.79)
	12	Streptococcus agalactiae	100 % (Ptrue = 82.54)
PREP-OPTIMA kit total efficiency	446	**100 % (Ptrue = 99.49)**	
* The amount of isolated genomic DNA was sufficient for further PCR studies			

b. Within-batch and between-batch precision

Within-batch precision – 100 % (86.28-100).

Between-batch precision - 100 % (86.28-100).

10. QUALITY CONTROL

"DNA-Technology Research&Production", LLC declares that the abovementioned products meet the provision of the Regulation (EU) 2017/746 of the European parliament and of the Council of 5 April 2017. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDR 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-OPTIMA DNA Extraction Kit.**

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11. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device	VER	Version
	Temperature limit		Date of manufacture
Σ	Contains sufficient for<n>tests		Consult instructions for use
	Use-by date	REF	Catalogue number
LOT	Batch code		Manufacturer
EC REP	Authorized representative in the European Community		Caution
	Do not use reuse		

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