DNA-TECHNOLOGY

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CE IVD

For professional use only

PREP-NA DNA/RNA Extraction Kit

PREP-NA PLUS DNA/RNA Extraction Kit

INSTRUCTION FOR USE

EC REP

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REF P-002/1EU

P-002/2EU

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1 INTENDED USE

The **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** are intended for DNA/RNA extraction from biological materials (see Table 1) for further analysis with reverse transcription (RNA) and/or polymerase chain reaction (DNA). In the **PREP-NA PLUS DNA/RNA Extraction Kit** the total volume of purified DNA/RNA is larger (300 µL) comparing to standard **PREP-NA DNA/RNA Extraction Kit** (50 µL) for more PCR tests.

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

Extraction Kit	**PREP-NA DNA/RNA Extraction Kit**	**PREP-NA PLUS DNA/RNA Extraction Kit**
Biological material	Blood plasma, saliva, phlegm, milk, urine, ejaculate, prostate fluid, cerebrospinal fluid, epithelial scrapes from posterior pharyngeal wall, urethra, cervical canal, posterior vaginal vault, smears and washouts from nasal and oropharyngeal cavities, faeces, material from dead and sick animals (smears and washouts from trachea, nasal cavity, pharyngeal cavity, cloaca; faeces; internal organs) etc.	Blood plasma, saliva, phlegm, milk, urine, ejaculate, prostate fluid, cerebrospinal fluid, epithelial swabs from posterior pharyngeal wall, urethra, cervical canal, posterior vaginal vault, smears and washouts from nasal and oropharyngeal cavities, etc.

When operating with reagent kits:

- **HBV PCR detection Kit**;
- **HCV PCR detection Kit**;
- **HCV Real-time PCR genotyping Kit**;
- **HIV PCR detection Kit**;
- **Acute viral respiratory infections Real-Time PCR Detection Kit**;
- **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit**;
- **Influenza A virus REAL-TIME PCR Detection Kit**;
- **Influenza B virus REAL-TIME PCR Detection Kit**;
- **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit;**
- **Influenza A virus (subtype H5N1) PCR detection Kit**

only **PREP-NA DNA/RNA Extraction Kit** must be used.

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-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit.**

The **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kits** 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-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** are based on DNA/RNA extraction with precipitation. In order to extract specific product nucleic acids (NA), the biological samples' cells are pretreated with NA extraction solution. The following steps for NA extraction are performed to prepare the lysed NA specimen for amplification.

3 CONTENT

The detailed description of content is represented in Tables 2-3.

Table 2. The **PREP-NA DNA/RNA Extraction Kit** content for P-002/1EU

Reagent	Description	Total volume	Amount
Lysis buffer	Light blue slightly foaming liquid	30 mL	1 vial
Precipitation buffer	Colorless transparent liquid	40 mL	1 vial
Washout solution №1	Colorless transparent liquid	50 mL	1 vial
Washout solution №2	Colorless transparent liquid	30 mL	1 vial
Dilution buffer	Colorless transparent liquid	5.0 mL (1.25 mL in each tube)	4 tubes
Negative control	Colorless transparent liquid	3.0 mL (1.5 mL in each tube)	2 tubes
Internal control (RNA-IC)	Colorless transparent liquid	1.0 mL	1 tube
Internal control (DNA-IC)	Colorless transparent liquid	1.0 mL	1 tube

Table 3. The **PREP-NA PLUS DNA/RNA Extraction Kit** content for P-002/2EU

Reagent	Description	Total volume	Amount
Lysis buffer	Light blue slightly foaming liquid	15 mL	1 vial
Precipitation buffer	Colorless transparent liquid	20 mL	1 vial
Washout solution №1	Colorless transparent liquid	25 mL	1 vial
Washout solution №2	Colorless transparent liquid	15 mL	1 vial
Dilution buffer	Colorless transparent liquid	15 mL	1 vial

Dilution buffer differs for **PREP-NA** and **PREP-NA PLUS** kits. Using of dilution buffer from another kit is not allowed.

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

The **PREP-NA DNA/RNA Extraction Kit** is designed for NA extraction from 100 analyzed samples (including negative controls). The **PREP-NA PLUS DNA/RNA Extraction Kit** is designed for NA extraction from 50 analyzed samples (including negative controls).

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 medium: "DNA-Technology" made **PREP-RAPID** (REF P-001/1EU) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU are recommended for **Acute viral respiratory infections Real-Time PCR Detection Kit**; **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit**; **Influenza A virus REAL-TIME PCR Detection Kit**; **Influenza B virus REAL-TIME PCR Detection Kit**; **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit Influenza A virus (subtype H5N1) PCR detection Kit)** or equivalent or physiological saline solution or sterile PBS of the sample 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. NA extraction

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 25-98 °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 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.

When extracting NA from phlegm (method 1):

- 10% trisodium phosphate x $12H_2O$;
- 1.0M HCl solution;
- 5.0% chloramine solution;
- Distilled water.

When extracting NA from phlegm (method 2):

- Mucolysin.

5 TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** must be stored at temperatures from 2 °C to 8 °C during the storage period.

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: the components of the kit should be stored at temperatures of 2 °C to 8 °C during the storage period.

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

An expired **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA 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-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA 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-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA 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, 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. 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-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** is designed to extract DNA/RNA from a wide variety of biological sample types, such as blood plasma, 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.

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 flacks 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 saline 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 sample 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.

Smears from the nasal cavity sampling

Take the smear with a dry sterile disposable swab into 1.5 mL plastic tubes with 300 mL of sterile physiological saline solution or a transport medium.

Order of taking:

1. Insert the swab carefully along the outer wall of the nose to a depth of 2-3 cm to the lower shell. Then lower the swab down slightly, insert into the lower nasal passage under the lower nasal conch, after a rotational movement remove along the outer wall of the nose.
2. Open the tube.
3. Put the swab into the tube with transport medium, rotate the swab for 10-15 seconds and rinse it thoroughly. Avoid spraying of solution.
4. Remove the swab from the solution and, by rotating it against the wall of the test tube above the level of the solution, squeeze out the excess liquid. Dispose the used swab.
5. Close the tube tightly and mark it.

Smears from the oropharynx sampling

Take the smears with a dry sterile disposable swab into 1.5 mL plastic tubes with 300 mL of sterile physiological saline solution or a transport medium.

Order of taking:

1. Take the smear with a swab with a rotational movement from the surface of the tonsils, palatine arches and the back wall of the pharynx.
2. Open the tube.
3. Put the swab into the tube with transport medium, rotate the swab for 10-15 seconds and rinse it thoroughly. Avoid spraying of solution.
4. Remove the swab from the solution and, by rotating it against the wall of the test tube above the level of the solution, squeeze out the excess liquid. Dispose the used swab.
5. Close the tube tightly and mark it.

Oropharyngeal lavage sampling

Before taking oropharyngeal lavage, rinsing the mouth with water should be done. After that, patient should rinse the oropharynx (for 10-15 seconds) with 8.0-10 mL of sterile physiological saline solution. Collect the liquid through a funnel into a sterile tube. Do not reuse the funnel without preliminary autoclaving. Transfer the lavage from the oropharynx (300 µL) into 1.5 mL plastic test tubes, close the test tube and mark.

Nasal cavity lavage sampling

Take the material in the patient's sitting position with the head tilted back. To obtain a nasal cavity lavage, inject 3.0-5.0 mL of warm sterile physiological saline solution alternately into both nasal passages using a swab or a disposable syringe. Collect the liquid through a funnel into a sterile tube. Do not reuse the funnel without preliminary autoclaving. Transfer the lavage from the oropharynx (300 µL) into 1.5 mL plastic test tubes, close the test tube and mark.

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 µL) to the sterile container and close it tightly.

Ejaculate, prostate fluid sampling

Put 100 µL of the liquid sample into the 1.5 mL tube with transport medium (or alternatively with 500 µL of sterile physiological saline solution).

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.

Faeces sampling

Put approximately 250 mg (µL) of faeces into the 1.5 mL tube with 1.0 mL of sterile physiological saline solution.

Animal internal organs sampling

Transfer ~250 mg analyzed material into 1.5 mL tubes.

Transportation and storage of the samples

Samples may be transported and stored in physiological saline solution at temperatures from 2 °C to 8° C no more than 24 hours prior to analysis. 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 no longer than 3 months.

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 blood

1. Centrifuge the tubes with blood at RCF(g) 900 for 20 minutes at the room temperature (from 18 °C to 25 °C).
2. Take the upper fraction (plasma) with a semi-automatic pipettes and put it into the new 1.5 mL tube.

The samples are ready for NA extraction.

Time from peripheral blood sampling to obtaining plasma must not exceed 6 hours. Plasma storage at minus 20 °C for not longer than 3 months is accepted.

Mix plasma just before NA extraction.

Preparation of the phlegm

Method 1:

1. Put approximately 500 µL 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 x12H_2O 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% solution of chloramine for disinfection.
6. Add 500 µL 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 µL (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 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 NA 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 RNA/DNA extraction necessity).

Preparation of the genitourinary epithelial scrapes

1. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
2. Remove the supernatant, leaving approximately 100 µL (precipitate+liquid fraction) in the tube.

The samples are ready for NA extraction.

Preparation of the smears and lavages

1. Centrifuge the tube with analyzed material at RCF(g) 16000 for 10 minutes.
2. Remove the supernatant leaving approximately 100 µL (pellet + liquid fraction).

The samples are ready for NA extraction.

Do not perform centrifugation and removal of the supernatant when testing samples for presence of infections caused by RNA-containing viruses. The sample is ready to RNA extraction.

Preparation of the urine

1. Transfer 1.0 mL of the sample into 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 physiological saline solution to the precipitate.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving approximately 100 µL (precipitate+liquid fraction) in the tube.

The samples are ready for NA extraction.

Preparation of the saliva, cerebrospinal fluid, synovial fluid

1. Transfer 500 µL of the sample into 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 physiological saline solution to the precipitate.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving approximately 100 µL (precipitate+liquid fraction).

The samples are ready for NA extraction.

Preparation of the ejaculate, prostate fluid

1. Vortex the tubes with samples for 5-10 seconds.
2. Centrifuge the tubes at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant, leaving approximately 100 µL (precipitate+liquid fraction) in the tube.

The samples are ready for NA extraction.

Preparation of the milk

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

The samples are ready for NA extraction.

Preparation of the faeces

1. Vortex the tube with sample for 5-10 seconds.
2. Centrifuge the tube at RCF(g) 100 for 2-3 minutes.
3. Transfer 800–1000 µL liquid material into 1.5 mL tube.
4. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
5. Remove the supernatant, leaving approximately 100 µL (precipitate+liquid fraction) in the tube.

The samples are ready for NA extraction.

Preparation of the animal internal organs

1. Add 1.0 mL sterile physiological saline solution into the tube with sample.
2. Vortex the tube for 3–5 seconds.
3. Centrifuge the tube at RCF(g) 1000 for 3-5 seconds.
4. Remove the supernatant.

The samples are ready for NA extraction.

8 PROCEDURE

Nucleic acid extraction

Simultaneously with the extraction of NA, a negative control sample should go through all stages of NA extraction.

Assay procedure:

The lysis buffer can form the precipitate. Dissolve it at 65 °C for 10 minutes prior to use.

8.1 Mark the required number of 1.5 mL tubes considering the number of samples to be tested and negative control (C-).

For pre-processed samples with obtaining pellet and supernatant (phlegm method 1, saliva, cerebrospinal fluid, urine, ejaculate, prostatic fluid, smears and lavages, faeces) tubes with 100 µL of material prepared for testing must be marked.

8.2 Add 10 µL of premixed internal control to each marked tube:

RNA-IC	DNA-IC
Reagent kits: **HCV PCR Detection Kit**; **HCV Real-time PCR Genotyping Kit; HIV PCR Detection Kit**; **Influenza A virus (subtype H5N1) PCR Detection Kit**; **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit**[1]	**HBV PCR Detection Kit**

1-In case of **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit**, RNA-IC from the **PREP-NA DNA/RNA Extraction Kit** is not used. Use RNA-IC from **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit**.

Do not use RNA-IC or DNA-IC at the sample preparation stage for the PCR kits which are not mentioned in the Table above.

Addition of both (RNA-IC and DNA-IC) an internal control is required when you do simultaneous analyses for presence of infections caused by RNA-containing viruses (**HCV** and **HIV**) and DNA-containing viruses (**HBV**).

8.3 Add 300 µL of the lysis buffer into the each tube avoiding contact of the pipette tip with an edge of the tube. 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 100 µL of the sample into the marked tubes (except for sample tubes passed preprocessing to obtain a precipitate (phlegm method 1, saliva, cerebrospinal fluid, urine, ejaculate, prostatic fluid, smears and lavages, faeces), and “C-” tube).

8.5 Add 100 µL of negative control (**PREP-NA** kit), transport medium or sterile physiological saline (**PREP-NA PLUS** kit) into the tube marked “C-”. Close the tube tightly, vortex for 3-5 seconds.

8.6 Incubate the tubes for 15 minutes at 65 °C, centrifuge at RCF(g) 16000 for 30 seconds.

In case of Influenza A virus RNA extraction from animal organ incubate the tubes at 65 °C for 30 minutes, spin down condensate for 3–5 seconds and remove the supernatant into new 1.5 mL tube.

8.7 Add 400 µL of the precipitation buffer. Close the tubes tightly and vortex them for 3–5 seconds.

At **HCV, HBV** and **HIV** NA extraction it is necessary to vortex the tubes twice.

8.8 Centrifuge the tubes at RCF(g) 16000 for 15 minutes.

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

8.10 Add 500 µL of the washout solution №1 to the precipitate and mix by inverting the tube 3-5 times.

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

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

8.13 Add 300 µL of the washout solution №2 to the precipitate and mix by inverting the tube 3-5 times.

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

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

8.16 Open the tubes and dry the precipitate at 65 °C for 5 minutes.

8.17 Add 50 µL (**PREP-NA** kit) or 300 µL **(PREP-NA PLUS** kit) dilution buffer to pellet (if it is necessary to increase the sensitivity of the study or if this is indicated in the detection kit user manual, the volume of the dilution buffer can be reduced).

Dilution buffer differs for **PREP-NA** and **PREP-NA PLUS** kits. Using of dilution buffer from another kit is not allowed.

Dissolve pellet in dilution buffer is recommended when testing clinical material for presence of infections caused by RNA-containing viruses alone (in 16.5 µL dilution buffer when working with **HCV PCR detection Kit**, **HCV Real-time PCR genotyping Kit**, **HIV PCR detection Kit**, in 35 µL dilution buffer when working with **Acute viral respiratory infections Real-Time PCR Detection Kit** and in 50 µL dilution buffer when working with **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit**; **Influenza A virus REAL-TIME PCR Detection Kit**; **Influenza B virus REAL-TIME PCR Detection Kit**; **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit)**, because dilution buffer volume increase leads to proportional sample dilution and decrease of analysis sensitivity. Dissolve pellet in 25 µL dilution buffer when working with **HBV** PCR detection kit and when simultaneously testing samples for presence of infections caused by RNA-containing viruses (**HCV** and **HIV**) and DNA-containing viruses (**HBV**).

8.18 Spin the drops down for 1-3 seconds.

8.19 Incubate the tubes at 65 °C for 10 minutes, vortex them for 3–5 seconds.

8.20 Centrifuge the tubes at RCF(g) 16000 for 30 seconds.

The DNA/RNA preparation is ready for reverse transcription and/or PCR.

The resulting RNA preparation must be used immediately for RT-PCR. If it is needed, the resulting RNA preparation can be stored at temperatures no higher than minus 18 °C for no longer than 7 days with a single defrost before reverse transcription.

DNA preparation can be stored at temperatures from minus 18 °C to minus 20 °C for no longer than 1 month or at temperatures from minus 68 °C to minus 70 °C for not longer than 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-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit**.

Technical support:

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10 KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code	VER	Version
CONTROL –	Negative control	NON STERILE	Non-sterile
EC REP	Authorized representative in the European Community		Caution

REF P-002/1EU

P-002/2EU

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