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For research use only

PREP-NA-ULTRA viral DNA/RNA Extraction Kit

INSTRUCTION FOR USE

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REF P-017-N/1EU

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TABLE OF CONTENTS

1. INTENDED USE

The **PREP-NA-ULTRA viral DNA/RNA Extraction Kit** is intended for virus nucleic acid isolation from blood plasma with preconcentration for subsequent real-time RT-PCR analysis.

The **PREP-NA-ULTRA viral DNA/RNA Extraction Kit** is an auxiliary agent for *in vitro* diagnostics in clinical and diagnostic laboratory.

Indications for the analysis: the need to isolate virus nucleic acids from blood plasma.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **PREP-NA-ULTRA viral DNA/RNA Extraction Kit.**

The **PREP-NA-ULTRA viral DNA/RNA Extraction Kit** can be used in research practice.

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

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

2. METHOD

The **method principle** is based on treating the sample with a multicomponent reagent that concentrates the nucleic acids from the primary sample volume, lysis of the resulting concentrate (precipitate), alcohol precipitation of the nucleic acids, washing and subsequent dissolution of the nucleic acids in buffer. The sample is then ready for PCR or RT-PCR.

Preconcentration of the sample increases the sensitivity of pathogen detection in clinical material.

3. CONTENT

The **PREP-NA-ULTRA viral DNA/RNA Extraction Kit** content is represented in Table 1.

Table 1. The **PREP-NA-ULTRA viral DNA/RNA Extraction Kit** content, package N for P-017-N/1EU

Reagent	Description	Total volume	Amount
Solution for concentration	Colorless transparent liquid*	80 mL	1 vial
Lysis solution	Light blue foamy liquid**	30 mL	1 vial
Precipitation buffer	Colorless transparent liquid	40 mL	1 vial
Wash solution No.1	Colorless transparent liquid	50 mL	1 vial
Wash solution No.2	Colorless transparent liquid	50 mL (25 mL in each vial)	2 vials
Dilution buffer	Colorless transparent liquid	10 mL	1 vial
Negative control	Yellow transparent or slightly opalescent liquid	42 mL	1 vial
* - Presence of precipitate that dissolves upon heating is allowed ** - Presence of crystals that dissolve upon heating is allowed			

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

The kit is intended for single use and designed for DNA extraction from 100 analyzed samples (including controls samples).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- For blood collection: 2.0 - 5.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of ethylenediaminetetraacetate (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-III;
- Refrigerator;
- Freezing chamber;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 1600) for Vacuette tubes;
- High speed centrifuge (RCF(g) no less than 14000) for 1.5 - 2.0 mL tubes;
- Solid-state thermostat (temperature range 24-65 °C);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Pipette stand;
- Tube rack for 1.5 mL tubes;
- Tube rack for 2.0 mL tubes;
- RNase and DNase free 1.5 mL tubes;
- RNase and DNase free 2.0 mL tubes;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

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

A precipitate that dissolves upon heating is allowed in solution for concentration.

Crystals that dissolve upon heating are allowed in lysis solution.

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

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

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

- Solution for concentration, lysis solution and wash solution No.2 should be stored at room temperatures (from 18 °C to 25 °C);
- Negative control and dilution buffer should be stored at temperatures from 2 °C to 8 °C during the storage period;
- Precipitation buffer and wash solution No.1 should be stored at temperatures from 2 °C to 8 °C during the storage period and used chilled.

ATTENTION! The lysis solution should be stored in a place protected from light.

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

An expired the **PREP-NA-ULTRA viral 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-ULTRA viral DNA/RNA 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, reverse transcription, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

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

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

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

Emergency actions

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

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

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

Do not use the kit:

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

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

7. SAMPLES

Blood plasma obtained from human peripheral whole blood is used for the assay.

Sample collection

Peripheral blood

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 -5.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL. The use of sodium citrate anticoagulant is also applicable. After taking the material, it is necessary to mix the blood with anticoagulant inverting the tube 2 – 3 times.

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

Transportation and storage of the samples

ATTENTION! The overall storage of the sample should not exceed 6 hours.

The transportation and storage temperature from collecting the sample until analysis should be from 2 °C to 8 °C.

ATTENTION! Whole blood cannot be frozen!

Sample preparation

1 Centrifuge the tube with blood at RCF(g) 800-1600 for 20 minutes at room temperatures from 18 °C to 25 °C.

2 Take the upper fraction (plasma) with a dispenser and put it into the new 1.5-2.0 mL tube.

The blood plasma can be stored at the temperature from minus 18 °C to minus 22 °C for no more than 3 months, at a temperature of minus 68 °C to minus 72 °C for no more than one year.

ATTENTION! Avoid repeated freezing and thawing of samples.

8. PROCEDURE

General requirements

To prevent cross-contamination of the biological material in the laboratory, the following rules are recommended:

- it is necessary to conduct a visual assessment of the incoming biomaterial and cull test tubes with broken integrity;
- it is necessary to use negative control samples, starting from the stage of RNA extraction in each protocol;
- use tips with aerosol filters at all stages of the assay;
- observe the assay procedure, open Eppendorf-type tubes without touching the inner part of the tube cap with gloved hand;
- do not touch the edge of the tube with the tip when adding reagents.

8.1 The use of control samples at the stage of nucleic acid extraction

8.1.1 Internal control sample

To exclude false negative results an internal control should be used during sample preparation.

An internal control can be added to clinical samples during the nucleic acid extraction step. The internal control is added to the clinical sample in the amount specified in the instructions for use of the corresponding PCR kit.

8.1.2 Negative control sample

To exclude false positive results and sample preparation assess use a negative control sample from the nucleic acid extraction stage.

8.1.3 Standards

Standards (according to the instructions for use of the corresponding set) can be used for quantitative tests. Standards can be used starting from the extraction step. Standards are prepared according to the instructions for use of the corresponding kit.

8.2 RNA extraction

ATTENTION!

1 Use DNAse and RNAse free filter tips;

2 Open only the cap of the tube which is in work, then close the tube before proceeding to the next tube to prevent contamination.

NOTE.

- Before starting work, warm the solution for concentration and lysis solution at 65 °C on the thermostat for 15 minutes. Then mix the solutions by turning the vial upside down 5-10 times, avoiding foaming;

- If the solution for concentration and lysis solution are stored at room temperature (18 °C to 25 °C), preheating is not necessary;
- Use the precipitation buffer and wash solution No.1 refrigerated for better visualization of the precipitate. For this purpose it is recommended to take them out of the refrigerator immediately before work;
- Thaw plasma samples stored at minus 18 °C or below at room temperature or 2 °C to 8 °C, mix on a vortex mixer, and centrifuge at RCF(g) no less than 14000 for 5 minutes. The supernatant must be used for the assay without touching the precipitate (if any);
- Mix plasma samples stored at 2 °C to 8 °C as well as control samples and standards on a vortex mixer and spin for 1-3 seconds on a vortex mixer.

8.2.1. Mark the required number of the 2.0 mL tubes:

- tubes for plasma test samples;
- one tube for negative control “C-“;
- three tubes for standard "ST1" (if specified in the instructions for use for the quantitative test kit);
- three tubes for standard "ST2" (if specified in the instructions for use for the quantitative test kit).

8.2.2. Add control samples and standards according to the instructions for use for the corresponding reagent kits.

When using reagent kits manufactured by “DNA-Technology”:

Add into the marked tubes (except for tubes "ST1" and "ST2"):

- for RNA isolation - 10 µL of RNA-IC ”A”;
- for DNA isolation - 10 µL of DNA-IC;
- in case of simultaneous RNA and DNA isolation - 10 µL of RNA-IC ”A” and 10 µL of DNA-IC.

8.2.3. Add 800 µL of solution for concentration to each tube.

NOTE. If the volume of test material is small, the reagent kit can be used to detect nucleic acids in 250 µL of sample. In this case, use a solution for concentration at the rate of 300 µL per 250 µL of sample.

8.2.4. Add 1000 µL of previously prepared blood plasma samples to the test tubes, close the tubes.

8.2.5. Add 1000 µL of negative control to the tube marked “C-“, close the tube.

8.2.6. When performing the quantitative analysis, place 20 µL of the appropriate standard and 980 µL of the negative control into tubes marked "ST1" and "ST2”. Close the tubes.

8.2.7. Shake the tubes on a vortex mixer for 1-3 seconds.

8.2.8. Centrifuge at RCF(g) 900 for 3 minutes at room temperature (18 °C to 25 °C).

8.2.9. Remove the supernatant completely from each tube using a separate tip without touching the sediment.

NOTE. In tubes with standards "ST1" and "ST2", negative control, as well as in some tubes with the test samples, the precipitate may not be visualized.

8.2.10. Add 300 µL of lysis solution to each tube without touching the edge of the tube; close the tubes.

8.2.11. Shake all tubes on a vortex mixer for 10-15 seconds.

8.2.12. Incubate the tubes at room temperature (18 °C to 25 °C) for 15 minutes until the precipitate is completely dissolved. During the incubation shake the tubes twice at 5 minutes intervals on a vortex mixer for 1-3 seconds.

NOTE. In the case of highly heterogeneous lysates, specify centrifugation parameters, since exceeding optimal RPM values may result in precipitates that are difficult to dissolve with lysis buffer.

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

8.2.14. Centrifuge the tubes at RCF(g) no less than 14000 for 30 seconds.

8.2.15. Add 400 µL of pre-cooled precipitation buffer to each tube.

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

8.2.17. Centrifuge the tubes at RCF(g) no less than 14000 for 10 minutes at room temperature (18 °C to 25 °C).

8.2.18. Remove the supernatant completely from each tube using a separate tip without touching the sediment.

8.2.19. Add 500 µL of wash solution No.1 to the precipitate, close the tube caps and gently invert each tube 3-5 times.

8.2.20. Centrifuge the tubes at RCF(g) no less than 14000 for 1 minute.

8.2.21. Remove the supernatant completely from each tube using a separate tip without touching the sediment.

8.2.22. Add 500 µL of wash solution No.2 to the precipitate, close the tube caps and gently invert each tube 3-5 times. It is necessary to invert each tube individually.

8.2.23. Centrifuge the tubes at RCF(g) no less than 14000 for 1 minute at room temperature (18 °C to 25 °C).

8.2.24. Remove the supernatant completely from each tube using a separate tip without touching the sediment.

8.2.25. Open the tube caps and dry the precipitate at 65 °C for 5 minutes.

8.2.26. Add 100 µL of dilution buffer to each tube with the precipitate. Close the tube caps.

8.2.27. Shake the tubes on a vortex mixer for 1-3 seconds.

8.2.28. Incubate the tubes in the thermostat at 65 °C for 10 minutes.

8.2.29. Shake the tubes on a vortex mixer for 1-3 seconds.

8.2.30. Centrifuge the tubes at RCF(g) no less than 14000 for 30 seconds.

The obtained RNA preparation must be used within 2 hours for the reverse transcription reaction and the polymerase chain reaction, since the RNA product cannot be stored.

DNA preparation can be stored at temperature from minus 18 °C to minus 22 °C for not longer than a month or at temperature from minus 68 °C to minus 72 °C for not longer than a year.

9. SPECIFICATIONS

a. The minimal amount of biomaterial for nucleic acids extraction is 250 µL.

Recommended amount of biomaterial to increase the sensitivity of the test: 1000 µL.

b. Effectiveness of the reagent kit

Number of samples (n) - 60;

Efficiency of HCV RNA isolation – 100% (97.8-100%).

Efficiency of HBV DNA isolation – 100% (97.8-100%).

10. 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 customer service with quality issues of the **PREP-NA-ULTRA viral DNA/RNA Extraction Kit**:

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11. KEY TO SYMBOLS

RUO	For research use only		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Keep away from sunlight
LOT	Batch code	VER	Version
	Manufacturer		Caution
	Non-sterile		

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