DNA-TECHNOLOGY

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For research use only

PREP-MB-LITE viral DNA/RNA Extraction Kit

INSTRUCTION FOR USE

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REF

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1. INTENDED USE

The **PREP-MB-LITE viral DNA/RNA Extraction Kit** is designed for viral nucleic acid extraction from blood plasma and blood serum for subsequent real-time PCR/RT-PCR analysis.

Indications for the analysis: the need to extract viral DNA/RNA from blood plasma, blood serum.

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

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

Potential users: qualified personnel trained in molecular research methods and rules of work in the laboratory.

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

2. METHOD

The **method principle** is lysis and extraction of nucleic acids under the influence of chaotropic agents, detergents and proteinase K with further sorption on paramagnetic particles, washing from impurities and elution with buffer.

3. CONTENT

The **PREP-MB-LITE viral DNA/RNA Extraction Kit** content is represented in Tables 1-4.

Table 1. The **PREP-MB-LITE viral DNA/RNA Extraction Kit** content, package A for P-136-A/9ER

Reagent	Description	Row No. in the cartridge	Number of cells/tubes	Reagent volume
Cartridge with reagents No. 1[1]				
Lysis solution	Colorless transparent foamy liquid	1[2]	2 cells	6.6 mL in each
Lysis solution	Colorless transparent foamy liquid	2	2 cells	6.6 mL in each
Wash solution No. 1	Green transparent liquid	3	2 cells	4.2 mL in each
Wash solution No. 1	Green transparent liquid	4	2 cells	4.2 mL in each
Elution solution	Colorless or pink transparent liquid	5	2 cells	2.7 mL in each
Wash solution No. 2	Yellow transparent liquid	6	2 cells	5.9 mL in each

[1] - The kit includes 2 cartridges with reagents No. 1 and 2 cartridges with reagents No. 2

[2] - row No. 1 of the cartridge with reagents has a side bevel

Cartridge with reagents No. 2[1]				
Wash solution No. 2	Yellow transparent liquid	1[2]	2 cells	5.9 mL in each
Wash solution No. 2	Yellow transparent liquid	2	2 cells	5.9 mL in each
Wash solution No. 2	Yellow transparent liquid	3	2 cells	3.0 mL in each
Wash solution No. 2	Yellow transparent liquid	4	2 cells	3.0 mL in each
Wash solution No. 2	Yellow transparent liquid	5	2 cells	5.9 mL in each
Wash solution No. 2	Yellow transparent liquid	6	2 cells	5.9 mL in each
Proteinase K	Colorless transparent viscous liquid	2 tubes		720 µL in each
Sorbent	Liquid with precipitate forming brown suspension upon shaking	4 tubes		480 µL in each
Dilution reagent	Blue transparent liquid	8 tubes		500 µL in each

Table 2. The **PREP-MB-LITE viral DNA/RNA Extraction Kit** content, package N for P-136-N/9ER

Reagent	**Description**	**Number of vials/tubes**	**Reagent volume**
Lysis solution	Colorless transparent foamy liquid	1 vial	53 mL
Proteinase K	Colorless transparent viscous liquid	2 tubes	720 µL in each
Sorbent	Liquid with precipitate forming brown suspension upon shaking	4 tubes	480 µL in each
Dilution reagent	Blue transparent liquid	1 vial	12 mL
Wash solution No. 1	Green transparent liquid	1 vial	34 mL
Wash solution No. 2	Yellow transparent liquid	2 vials	75 mL in each
Elution solution	Colorless or pink transparent liquid	1 vial	11 mL

Table 3. The **PREP-MB-LITE viral DNA/RNA Extraction Kit** content, package P, Set No. 1 for P-136-P/9ER

Reagent	**Description**	**Row No. in the cartridge**	**Number of cells/tubes**	**Reagent volume**
Cartridge with reagents				
Lysis solution	Colorless transparent foamy liquid	1[3]	2 cells	6.6 mL in each
Lysis solution	Colorless transparent foamy liquid	2	2 cells	6.6 mL in each
-	-	3	-	-
-	-	4	-	-
Lysis solution	Colorless transparent foamy liquid	5	2 cells	6.6 mL in each
Lysis solution	Colorless transparent foamy liquid	6	2 cells	6.6 mL in each
Wash solution No. 1	Green transparent liquid	1 plate		96 wells (350 µL in each)
Wash solution No. 2	Yellow transparent liquid	1 plate		96 wells (730 µL in each)
Elution solution	Colorless or pink transparent liquid	1 plate		96 wells (110 µL in each)
Proteinase K	Colorless transparent viscous liquid	2 tubes		720 µL in each
Sorbent	Liquid with precipitate forming brown suspension upon shaking	4 tubes		480 µL in each
Dilution reagent	Blue transparent liquid	4 tubes		500 µL in each

[3] - row No. 1 of the cartridge with reagents has a side bevel

Table 4. The **PREP-MB-LITE viral DNA/RNA Extraction Kit** content, package P, Set No. 2 for P-137-P/9ER

Reagent	Description	Row No. in the cartridge	Number of cells/tubes	Reagent volume
Cartridge with reagents				
Lysis solution	Colorless transparent foamy liquid	1[4]	2 cells	6.6 mL in each
Lysis solution	Colorless transparent foamy liquid	2	2 cells	6.6 mL in each
-	-	3	-	-
-	-	4	-	-
Lysis solution	Colorless transparent foamy liquid	5	2 cells	6.6 mL in each
Lysis solution	Colorless transparent foamy liquid	6	2 cells	6.6 mL in each
Wash solution No. 1	Green transparent liquid	1 plate		96 wells (350 µL in each)
Wash solution No. 2	Yellow transparent liquid	1 plate		96 wells (730 µL in each)
Elution solution	Colorless or pink transparent liquid	1 plate		96 wells (110 µL in each)
Proteinase K	Colorless transparent viscous liquid	2 tubes		720 µL in each
Sorbent	Liquid with precipitate forming brown suspension upon shaking	4 tubes		480 µL in each
Dilution reagent	Blue transparent liquid	4 tubes		500 µL in each
96 tip comb		1 pc		
96 deep-well plate		2 pcs		
PCR seal sheet		1 pc		

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

The kit in package A is designed for NA extraction from 96 test samples (one run of 96 samples or two runs of 48 samples each), including standards and negative controls. In case of quantitative test, NA extraction from 88 test samples is allowed (40 samples per run in case of two independent extractions). In case of qualitative test, NA extraction from 94 test samples is allowed (46 samples per run in case of two independent extractions).

The kit in package N (extraction in 1.5-2.0 mL tubes) is designed for NA extraction from 96 test samples, including standards and negative controls. In case of quantitative test, the manufacturer guarantees that the reagents are sufficient for 6 independent extractions, 9 test samples each. In case of the qualitative test, the manufacturer guarantees that the reagents are sufficient for 6 independent extractions, 15 test samples each.

The kit in package N (extraction in deep-well plates) is designed for NA extraction from 96 test samples (one run of 96 samples), including standards and negative controls. In case of quantitative test, NA extraction from 88 test samples is allowed. In case of qualitative test, NA extraction from 94 test samples is allowed.

The kit in package P is designed for NA extraction from 96 test samples (one run of 96 samples), including standards and negative controls. In case of quantitative test, NA extraction from 88 test samples is allowed. In case of qualitative test, NA extraction from 94 test samples is allowed.

[4] - row No. 1 of the cartridge with reagents has a side bevel

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

The following equipment, reagents and consumables are required:

Equipment, reagents and consumables	Package A	Package N, extraction in 1.5 mL tubes	Package N, extraction in deep-well plates	Package P
Biological safety cabinet class II	yes	yes	yes	yes
Solid-state thermostat, maintaining up to 65 °C	yes	yes	yes	yes
High speed centrifuge (RCF(g) at least 1,600) for Vacuette tubes	yes	yes	yes	yes
Dosing instrument DTstream 12L4 or 15L4[1] or Automatic module analyzer Freedom EVO with applications, Freedom EVO Clinical version	yes[2]	no	no	yes[2]
Centrifuge with rotor for deep-well plates, e.g. LMC-12, Biosan	yes	no	no	yes
Automatic extraction system KingFisher Flex 96 (Thermo Fisher Scientific Oi, Finland) or Automatic extraction system Auto-Pure 96 (Allsheng, China)	yes	no	yes	yes
Vortex mixer	yes	yes	yes	yes
Refrigerator with freezer	yes	yes	yes	yes
Single channel pipettes (dispensers covering 20-1,000 µL volume range)	yes	yes	yes	yes
RNase and DNase free filter pipette tips (volume 200 µL, 1,000 µL)	yes	yes	yes	yes
Pipette rack	yes	yes	yes	yes
Powder-free surgical gloves;	yes	yes	yes	yes
RNase and DNase free 1.5-2.0 mL tubes (SSI-1260 tubes are recommended)	no	yes	no	no
Tube rack for 1.5 mL tubes;	no	yes	no	no
Physiological saline solution (0.9% NaCl)	yes	yes	yes	yes
Container for used pipette tips and other consumables	yes	yes	yes	yes
Container for used DTstream pipette tips	yes[2]	no	no	yes[2]

Replaceable funnel for waste for DTstream	yes[2]	no	no	yes[2]
RNase and DNase free filter pipette tips (volume 1,000 µL) for DTstream or similar dosing device	yes[2]	no	no	yes[2]
96-well deep-well plate	yes	no	yes	yes
Adhesive plate-sealing film	yes	no	yes	yes
96 tip comb	yes	no	yes	yes[3]
Disinfectant solution	yes	yes	yes	yes
Electric laboratory aspirator with trap flask for the removal of supernatant	no	yes	no	no
RNase and DNase free pipette tips for aspirator with trap flask	no	yes	no	no
Magnetic rack	no	yes	no	no
Mechanical stepper 1.0-10 µL, e.g. Eppendorf Multipette M4; Syringe tips for stepper (5.0 mL and 1.0 mL), e.g. Combitips Advanced or Eight channel pipettes (dispensers covering 100-1,000 µL volume range), e.g. Biohit Proline	no	no	yes	yes[4]
Additionally for obtaining and preparation of blood plasma/serum:				
4.0 mL Vacuette plastic tubes with EDTA salt and sodium citrate	yes	yes	yes	yes
Centrifuge for Vacuette tubes, RCF(g) at least 800	yes	yes	yes	yes
Dosing instrument DTstream 12L1 or 15L1[5]	yes	no	no	yes

Notes:

[1] – calibration by service engineer is required at first run of the dosing instrument and when changing the 1.5 mL tube type

[2] – for automatic test sample addition using DTstream

[3] – except package P, Set No. 2 (P-137-P/9ER);

[4] – for manual deep-well plate preparation

[5] – optional

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

5.1. Storage conditions

- All components of the **PREP-MB-LITE viral DNA/RNA Extraction Kit,** except for proteinase K, must be stored in a refrigerator or a cooling chamber at the temperature from 2 °C to 25 °C throughout the shelf life of the kit. Cartridges with reagents, dilution reagent, lysis solution, wash solution No. 1, wash solution No. 2 and elution solution must be stored out of light throughout the shelf life of the kit.
- Proteinase K must be stored in a freezer at the temperature from minus 22 °C to minus 18 °C throughout the shelf life of the kit.
- The kit must be stored in the upright position in accordance with the handling sign “UP”.

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

5.2. Transport conditions

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

- It is allowed to transport the kit, except for proteinase K, in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container from 2 °C to 25 °C for no longer than 5 days.
- It is allowed to transport proteinase K in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container up to 25 °C for no longer than 5 days.
- The kit must be transported in the upright position in accordance with the handling sign “UP”.

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

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

- All components of the kit, except for proteinase K, must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 25 °C over the storage period. Cartridges with reagents, dilution reagent, lysis solution, wash solution No. 1, wash solution No. 2 and elution solution must be stored out of light over the storage period.
- Proteinase K must be stored in a freezer at temperatures from minus 22 °C to minus 18 °C over the storage period.
- The kit must be stored in the upright position in accordance with the handling sign “UP”.

WARNING! The kits stored under undue regime must not be used.

An expired **PREP-MB-LITE viral DNA/RNA 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-MB-LITE 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 research and the rules of work in the laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the test 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 test. 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 and blood serum obtained from human peripheral whole blood is used for the test.

Note. If using the kit together with **HCV Quantitative REAL-TIME PCR Kit** and **HCV REAL-TIME PCR Kit** (made by "DNA-Technology"), we recommend to use blood plasma for the test.

7.1. General requirements

- The quality of sampling, sample storage, transport and pretreatment are of great importance for obtaining correct results. Incorrect sampling may lead to unreliable results and, therefore, to the necessity for repeated sampling.
- Use RNase and DNase free filtered tips during biomaterial preparation and NA extraction.
- To prevent contamination, only open the cap of the tube you are working with and close it before proceeding to the next tube.

7.2. Interfering substances

The presence of PCR inhibitors in a sample may cause controversial (uncertain) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

The maximum concentration of interfering substances, which do not affect the reverse transcription and amplification of the laboratory control and internal control: triglycerides – up to 40 mmol/L of plasma/serum sample, hemoglobin – up to 2.0 g/L, bilirubin – up to 340 µmol/L, total protein – 80 g/L, whole blood – 10% v/v.

Concentrations of exogenous substances in biomaterial (blood plasma/serum) not interfering the RT-PCR test: peg-interferon alpha, acyclovir, atazanavir, ribavirin, rifampicin, isoniazid, azithromycin – up to 3 maximum therapeutic concentrations.

7.3. Peripheral blood collection

WARNING! Preparation of peripheral blood samples before NA extraction is required!

Method limitations: intravenous heparin injections, infusion of parenteral nutrition less than 6 hours before the test.

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 4.0 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 (if it does not

contradict the requirements to the PCR/RT-PCR reagent kit used together with the **PREP-MB-LITE viral DNA/RNA Extraction Kit**). After taking the material, it is necessary to mix the blood with anticoagulant inverting the tube 2 – 3 times.

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

Using tubes with clot activator and barrier gel is allowed.

Fill the tube until the filling mark. This guarantees the right blood/anticoagulant (or clot activator) proportion in the tube and ensures a correct result. 10% deviation of sample volume in the tube is allowed.

7.4. Transportation and storage of the samples

WARNING! 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 for up to 12 hours.

WARNING! Whole blood cannot be frozen!

Note. It is not recommended to transport and store tubes with plasma at temperature below 0 °C to avoid gel damage and sample contamination with hemolysis products and gel fragments.

7.5. Obtaining blood plasma/serum

Centrifuge the tube with blood at RCF(g) 800-1,600 for 20 minutes at room temperature from 18 °C to 25 °C.

Tubes with serum and barrier gel must be centrifuged once to separate the serum.

WARNING!

1. If using tubes with barrier gel, second centrifugation is prohibited to avoid gel damage and sample contamination with hemolysis products.
2. Avoid gel getting into the serum sample as it may result in PCR/RT-PCR inhibition.

If prolonged (>12 hours) storage and archiving of plasma/serum is not expected:

- for Freedom EVO® automatic dosing instruments, prepared (centrifuged) tubes with whole blood and with serum and battier gel can be used directly for NA extraction. To do this, before starting the extraction, store the tubes with whole blood and the tubes with serum vertically in racks, avoiding tilting and turning over;
- for DTstream automatic dosing instruments, transfer of plasma and serum from primary tubes with whole blood abd barrier gel into new Vacuette tubes us required and can be done either manually with a pipette or using DTstream 12L1 or 15L1;
- for manual NA extraction, plasma and serum are transferred into 1.5-2.0 mL plastic tubes with a pipette.

If blood plasma storage and archiving is expected, after centrifugation collect upper fraction (plasma/serum) with a pipette. Do not touch barrier gel (if any). Transfer into a 4.0 mL Vacuette tube.

Blood plasma/serum can be stored at temperature from 2 °C to 8 °C for no longer than 5 days, at minus 22 °C to minus 18 °C for no longer than 3 months, at minus 72 °C to minus 68 °C for no more than one year.

WARNING!

1. Avoid repeated freezing and thawing of samples.
2. Avoid barrier gel getting into the serum sample.

8. PROCEDURE

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

1. Visual assessment of the incoming biomaterial and cull test tubes with broken integrity are necessary.
2. it is necessary to use negative control samples, starting from the stage of NA extraction in each protocol.
3. Use tips with aerosol filters at all stages of the test.
4. Observe the procedure, open Eppendorf-type tubes using tweezers (avoid 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 (if this happened, change the tip immediately).

8.1 Preparation of plasma/serum samples for NA extraction

Preparation of plasma/serum samples for NA extraction is carried out according to Table 1.

Table 5. Orders of plasma/serum samples preparation

Storage temperature for plasma/serum samples	Preparation of plasma/serum samples
Minus 18 °C and below	1. Thaw plasma/serum samples at room temperature (18 °C to 25 °C) or at 2 °C to 8 °C. 2. Shake plasma/serum samples on vortex and centrifuge at RCF(g) 14,000 for 5 minutes.
	WARNING! 1. For NA extraction use supernatant. Do not touch the precipitate (if any). 2. If dosing instruments are to be used (DTstream/ Freedom EVO®), centrifuge at low RCF(g) (800-1,000), as any precipitate after centrifugation may block automatic plasma collection.
From 2 °C to 8 °C	Shake plasma/serum samples on vortex and spin on vortex for 1-3 seconds.

8.2 Use of controls during nucleic acid extraction

Using controls at NA extraction stage is prescribed by instructions for use to the PCR/RT-PCR reagent kits.

To exclude false negative results and for quality control, using internal control is recommended.

8.2.1 Internal control

An **internal control** must be added to test samples during NA extraction stage. Internal control is added to the test sample in the amount specified in the instructions for use of the corresponding PCR/RT-PCR kit.

8.2.2 Negative control

To exclude false positive results and sample preparation assess use a **negative control** from the NA extraction stage, simultaneously with NA extraction from test samples.

8.2.3 Standards

Standards (according to the instructions for use of the corresponding PCR/RT-PCR kit) can be used for quantitative tests. Standards can be used starting from NA extraction stage, simultaneously with NA extraction from test samples. Standards are prepared according to the instructions for use of the corresponding PCR/RT-PCR kit.

WARNING!

1. Use only RNase and DNase free filter tips at NA extraction stage, except for the supernatant collection with aspirator. In this case, use RNase and DNase free tips without filter.
2. Change the tip after each solution removal from the tube.
3. To prevent contamination, only open the cap of the tube you are working with and close it before proceeding to the next tube.
4. Be careful when adding solution to the tube with biomaterial. Do not touch the walls of the tube. If touching occurred, change the tip.

8.3 Preparation and NA extraction using package N and magnetic rack

WARNING! Before starting work:

1. Heat thermostat to 65 °C.
2. Tube caps may open during heating! Use tubes with locking caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with a clamp lid (e.g. solid-state programmable small-size thermostat TT-1-DNA-Technology, manufactured by "DNA-Technology R&P", LLC.).

8.3.1. Mark the required number of 1.5-2.0 mL tubes:

- one tube for each plasma/serum sample;
- one tube for negative control "C-".

For quantitative tests using **reagent kits manufactured by "DNA-Technology"**[5], mark additionally:

- 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).

WARNING! The reagents are intended for up to 6 separate extraction procedures considering variable number of test samples and 1 negative control "C-" per run. If the obtained NA is used for quantitative tests and the use of standards is prescribed by the quantitative reagent kit instruction, 3 additional tubes for ST1 and 3 tubes for ST2 are allowed in each run.

8.3.2. Add controls and standards according to the instructions for use for the corresponding reagent kits.

When using **reagent kits manufactured by "DNA-Technology"**, add into all the marked tubes:

- for RNA extraction – 20 µL of RNA-IC "A";
- for DNA extraction – 20 µL of DNA-IC "A";
- in case of simultaneous RNA and DNA extraction – 20 µL of RNA-IC "A" and 20 µL of DNA-IC "A".

Note. For small amounts of biomaterial, NA extraction from 100 µL of sample is possible.

8.3.3. Mix the tubes with sorbent and proteinase K on vortex for 3-5 seconds and spin on vortex for 1-3 seconds.

8.3.4. Add 550 µL of concentration solution (**the solution is colorless**), 15 µL of proteinase K and 20 µL of sorbent into each tube. Do not touch the walls of the tubes. Close the tubes.

8.3.5. Enter 250 µL or 100 µL of prepared samples of blood plasma/serum into the corresponding marked tubes. Close the tubes. Do not enter plasma/serum samples into the "C-", "ST1", "ST2" tubes.

[5] - If using reagent kits from other manufacturers, please refer to the instructions for use to the corresponding kits.

8.3.6. Turn the vial with dilution reagent upside down 5-10 times. Mix the standards on vortex and spin on vortex for 1-3 seconds.

8.3.7. Add 250/100 µL of dilution reagent into the "C-" tube. Close the tube.

8.3.8. For quantitative tests using **reagent kits manufactured by "DNA-Technology"**[7], enter 20 µL of the corresponding standard and 230/80 µL of dilution reagent into the "ST1" and "ST2" tubes (3 tubes per each standard). Close the tubes.

N o t e . In case of quantitative test for several infections, preparation of mixed standards is allowed. To make it, add 20 µL of ST1 from each reagent kit into each tube for ST1; add 20 µL of ST2 from each reagent kit into each tube for ST2. Then, reach the final volume of 250/100 µL by adding dilution reagent.

E x a m p l e :

1. Extraction from 250 µL:

If quantitative HCV and HBV tests are planned in one run, add 20 µL of HCV-ST1 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST1 from the **HBV Quantitative REAL-TIME PCR Kit** into ST1 tubes; add 20 µL of HCV-ST2 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST2 from the **HBV Quantitative REAL-TIME PCR Kit** into ST2 tubes. Then, add 210 µL of dilution reagent into each tube.

2. Extraction from 100 µL:

If quantitative HCV and HBV tests are planned in one run, add 20 µL of HCV-ST1 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST1 from the **HBV Quantitative REAL-TIME PCR Kit** into ST1 tubes; add 20 µL of HCV-ST2 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST2 from the **HBV Quantitative REAL-TIME PCR Kit** into ST2 tubes. Then, add 60 µL of dilution reagent into each tube.

8.3.9. Shake the tubes on vortex for 10-15 seconds.

8.3.10. Incubate the tubes at room temperature (18 °C to 25 °C) for 15 minutes. We recommend to shake the tube once on vortex for 1-3 seconds during incubation.

8.3.11. Spin the tubes on vortex and place them into a magnetic rack for 1.5-2 minutes until full separation of sorbent particles.

8.3.12. Remove supernatant completely using aspirator (separate tip without filter for each tube). Do not touch the sorbent.

8.3.13. Leaving the tubes in the rack, add 350 µL of wash solution No. 1 (**the solution is green**). Close the tubes.

8.3.14. Turn the tubes upside down for 3-5 seconds.

8.3.15. Spin the tubes on vortex for 3-5 seconds and place into magnetic rack for 1.5-2 minutes until full separation of sorbent particles.

8.3.16. Remove supernatant completely using aspirator (separate tip without filter for each tube). Do not touch the sorbent.

8.3.17. Leaving the tubes in the rack, add 750 µL of wash solution No. 2 (**the solution is yellow**). Close the tubes.

8.3.18. Turn the tubes upside down for 3-5 seconds.

8.3.19. Spin the tubes on vortex for 3-5 seconds and place into magnetic rack for 1.5-2 minutes until full separation of sorbent particles.

8.3.20. Remove supernatant completely using aspirator (separate tip without filter for each tube). Do not touch the sorbent.

8.3.21. Repeat 8.3.17-8.3.20 (wash the particles with wash solution No. 2).

8.3.22. Leaving the tubes in the rack, add 100 µL of elution solution (**the solution is colorless or pink**). Close the tubes.

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

8.3.24. Heat the tubes in thermostat at 65 °C for 10 minutes.

8.3.25. Shake the tubes on vortex for 1-3 seconds.

8.3.26. Spin down the drops on vortex for 30 seconds.

8.3.27. Place the tubes into the magnetic rack.

Supernatant contains purified DNA or RNA.

NA preparation is ready for PCR/RT-PCR. We recommend to leave the tubes with NA preparation in the magnetic rack to prevent the sorbent getting into the PCR-mix.

8.4 Preparation and NA extraction using package N and automatic DWP NA extraction systems

8.4.1. Prepare an automatic extraction system AutoPure-96 or KingFisher Flex 96 according to their operation manuals.

8.4.2. Prepare an empty deep-well plate (DWP) with tip comb.

8.4.3. Mark 5 new 96-well DWPs:
- No. 1 – for test samples, lysis solution and sorbent (LB),
- No. 2 – for wash solution No. 1 (WB1),
- No. 3 – for wash solution No. 2 (WB2),
- No. 4 – for wash solution No. 2 (WB2),
- No. 5 – for elution solution (EB).

WARNING! The reagents are intended for up to one independent extraction procedure, given:
- 94 test samples and negative control "C-" in 2 repeats in a run for further qualitative tests;
- 88 test samples and negative control "C-" in 2 repeats, ST1 in 3 repeats and ST2 in 3 repeats in a run for further quantitative tests.

8.4.4. Add controls and standards according to the instructions for use for PCR/RT-PCR kits.

When using **reagent kits manufactured by "DNA-Technology"**, add[6] into each well of DWP No. 1 (LB):

- for RNA extraction – 20 µL of RNA-IC "A";
- for DNA extraction – 20 µL of DNA-IC "A";
- in case of simultaneous RNA and DNA extraction – 20 µL of RNA-IC "A" and 20 µL of DNA-IC "A".

Note. For small amounts of biomaterial, NA extraction from 100 µL of sample is possible.

8.4.5. Mix the tubes with sorbent and proteinase K on vortex for 3-5 seconds and spin on vortex for 1-3 seconds.

8.4.6. Add 550 µL of lysis solution (**the solution is colorless**), 15 µL of proteinase K and 20 µL of sorbent into each well of DWP.

8.4.7. Add 250/100 µL of test samples into the corresponding wells of DWP No. 1.

8.4.8. Turn the vial with dilution reagent upside down 5-10 times. Mix the standards on vortex and spin on vortex for 1-3 seconds.

8.4.9. Add 250/100 µL of dilution reagent (depending on the sample volume) into the well for C-.

[6] - If this is prescribed by the PCR/RT-PCR reagent kit instruction for use

8.4.10. If quantitative test using **reagent kits manufactured by "DNA-Technology"** [7] is expected, add 20 µL of the corresponding standard and 230/80 µL of dilution reagent (depending on the sample volume) into the wells for ST1 and ST2 (3 wells for each standard).

N o t e . In case of quantitative test for several infections, preparation of mixed standards is allowed. To make it, add 20 µL of ST1 from each reagent kit into each well for ST1; add 20 µL of ST2 from each reagent kit into each well for ST2. Then, reach the final volume of 250/100 µL by adding dilution reagent into the well.

Example:

1. Extraction from 250 µL:

If quantitative HCV and HBV tests are planned in one run, add 20 µL of HCV-ST1 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST1 from the **HBV Quantitative REAL-TIME PCR Kit** into ST1 tubes; add 20 µL of HCV-ST2 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST2 from the **HBV Quantitative REAL-TIME PCR Kit** into ST2 tubes. Then, add 210 µL of dilution reagent into each well.

2. Extraction from 100 µL:

If quantitative HCV and HBV tests are planned in one run, add 20 µL of HCV-ST1 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST1 from the **HBV Quantitative REAL-TIME PCR Kit** into ST1 tubes; add 20 µL of HCV-ST2 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST2 from the **HBV Quantitative REAL-TIME PCR Kit** into ST2 tubes. Then, add 60 µL of dilution reagent into each well.

8.4.11. Add 350 µL of wash solution No. 1 (**the solution is green**) into each well of DWP No. 2 (WB1).

8.4.12. Add 730 µL of wash solution No. 2 (**the solution is yellow**) into each well of DWPs No. 3 and No. 4 (WB2).

8.4.13. Add 110 µL of elution solution (**the solution is colorless or pink**) into each well of DWP No. 5 (EB).

8.4.14. Install all the DWPs into the corresponding slots of Auto-Pure 96 or KingFisher Flex 96. Select and run NA extraction script.

8.4.15. Wait for the automatic NA extraction to finish.

NA preparation is ready for PCR/RT-PCR (is in DWP No. 5 with elution solution). To store the plate with NA, use adhesive sealing film[8].

8.5 Preparation and NA extraction using package A, DTstream dosing instrument and automatic DWP NA extraction systems

WARNING! We recommend to store cartridges with reagents at room temperature (18 °C to 25 °C) to avoid crystal formation. In case the kit has been stored at minus 18 °C and below, hold the cartridges at room temperature (18 °C to 25 °C) for at least 4 hours.

8.5.1 Prepare DTstream 15L4 or DTstream 12L4 for work according to the operation manual.

8.5.2 Prepare an automatic extraction system AutoPure-96 or KingFisher Flex 96 according to their operation manuals.

8.5.3 Prepare an empty deep-well plate (DWP) with tip comb.

[7] - If using reagent kits from other manufacturers, please refer to the instructions for use to the corresponding kits

[8] - Not included in the kit

8.5.4 **Sample aliquoting**

8.5.4.1 Mark 5 new 96-well DWPs:

- No. 1 – for test samples, lysis solution and sorbent (LB),
- No. 2 – for wash solution No. 1 (WB1),
- No. 3 – for wash solution No. 2 (WB2),
- No. 4 – for wash solution No. 2 (WB2),
- No. 5 – for elution solution (EB).

WARNING! The reagents are intended for up to one independent extraction procedure, given:

- 94 test samples and negative control "C-" in 2 repeats in a run for further qualitative tests;
- 88 test samples and negative control "C-" in 2 repeats, ST1 in 3 repeats and ST2 in 3 repeats in a run for further quantitative tests.

or for two independent extraction procedures, given:

- 46 test samples and negative control "C-" in 2 repeats in a run for further qualitative tests;
- 40 test samples and negative control "C-" in 2 repeats, ST1 in 3 repeats and ST2 in 3 repeats in a run for further quantitative tests.

8.5.4.2 Unpack racks with RNase and DNase free filter tips, remove the caps and install the racks onto the DTstream working table.

8.5.4.3 Install cartridge No. 1 ad cartridge No. 2 onto the DTstream working table with the bevelled corner forward, remove the protective cover.

WARNING! Do not take off the foil from the cartridges and do not pierce it.

8.5.4.4 Install the tubes with RNA-IC "A" or DNA-IC "A" [9], proteinase K and dilution reagent into the rack for reagents. If quantitative test using **reagent kits manufactured by "DNA-Technology"**[10] is expected, install the tubes with ST1 and ST2 into the rack for reagents.

8.5.4.5 Install DWPs No. 1 (LB), No. 2 (WB1), No. 3 and No. 4 (WB2), No. 5 (EB) onto the DTstream working table.

8.5.4.6 Install the tubes with test samples into the rack for test samples on the DTstream working table.

8.5.4.7 Prepare a container for waste according to the DTstream operation manual.

8.5.4.8 Check the readiness of DTstream:

- tip dispenser;
- the reagents, consumables and samples are installed in accordance with the working table scheme.

8.5.4.9 Using DTstream menu or a controlling device (see operation manual), run the dosing script. Select the sample volume for NA extraction – 250 µL or 100 µL. The dosing instrument will run automatic aliquoting of the lysis solution, internal controls, proteinase K and sorbent; preparation of negative controls, standards and introducing test samples into the DWP.

8.5.5 **DWP preparation**

8.5.5.1 Using DTstream menu or a controlling device (see operation manual), run the DWP preparation script.
The dosing instrument will run automatic aliquoting of wash solution No. 1 (**green**), wash solution No. 2 (**yellow**) and elution solution (**colorless or pink**).

[9] - If this is prescribed by the PCR/RT-PCR reagent kit instruction for use.
[10] - If using reagent kits from other manufacturers, please refer to the instructions for use to the corresponding kits.

8.5.5.2 Wait for the aliquoting and DWP preparation to finish, then install all the place into the corresponding slots of AutoPure-96 or KingFisher Flex 96. Select and run NA extraction script.

8.5.5.3 Wait for the automatic NA extraction to finish.

NA preparation is ready for PCR/RT-PCR (is in DWP No. 5 with elution solution). To store the plate with NA, use adhesive sealing film [11].

8.6 Preparation and NA extraction using package A, Freedom EVO® (TECAN) dosing instrument and automatic DWP NA extraction systems

WARNING! We recommend to store cartridges with reagents at room temperature (18 °C to 25 °C) to avoid crystal formation. In case the kit has been stored at minus 18 °C and below, hold the cartridges at room temperature (18 °C to 25 °C) for at least 4 hours.

8.6.1 Prepare Freedom EVO® for work according to the operation manual.

8.6.2 Prepare an automatic extraction system AutoPure-96 or KingFisher Flex 96 according to their operation manuals.

8.6.3 Prepare an empty deep-well plate (DWP) with tip comb.

8.6.4 **Sample aliquoting**

8.6.4.1. Mark 5 new 96-well DWPs and install them onto the Freedom EVO® working table according to the scheme:

- No. 1 – for test samples, lysis solution and sorbent (LB),
- No. 2 – for wash solution No. 1 (WB1),
- No. 3 – for wash solution No. 2 (WB2),
- No. 4 – for wash solution No. 2 (WB2),
- No. 5 – for elution solution (EB).

WARNING! The reagents are intended for up to one independent extraction procedure, given:

- 94 test samples and negative control “C-“ in 2 repeats in a run for further qualitative tests;
- 88 test samples and negative control “C-“ in 2 repeats, ST1 in 3 repeats and ST2 in 3 repeats in a run for further quantitative tests;

or for two independent extraction procedures, given:

- 46 test samples and negative control “C-“ in 2 repeats in a run for further qualitative tests;
- 40 test samples and negative control “C-“ in 2 repeats, ST1 in 3 repeats and ST2 in 3 repeats in a run for further quantitative tests.

8.6.4.2. Unpack racks with RNase and DNase free filter tips, remove the caps and install the racks onto the Freedom EVO® working table.

8.6.4.3. Install cartridges No. 1 and No. 2 onto the Freedom EVO® working table with the bevelled corner forward, remove the protective cover.

WARNING! Do not take off the foil from the cartridges and do not pierce it.

8.6.4.4. Install DWPs No. 1 (LB), No. 2 (WB1), No. 3 and No. 4 (WB2), No. 5 (EB) onto the Freedom EVO® working table.

8.6.4.5. Install the tubes with RNA-IC “A” or DNA-IC “A” [12], proteinase K and dilution reagent, into the rack for reagents. If quantitative test using **reagent kits manufactured by “DNA-Technology”**[13] is expected, install the tubes with ST1 and ST2 into the rack for reagents.

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[11] - Not included in the kit

[12] - If this is prescribed by the PCR/RT-PCR reagent kit instruction for use.

[13] - If using reagent kits from other manufacturers, please refer to the instructions for use to the corresponding kits.

8.6.4.6. Install the tubes with test samples into the rack for test samples on the Freedom EVO® working table.

8.6.4.7. Check the readiness of Freedom EVO®:

- tip dispenser;
- the reagents, consumables and samples are installed in accordance with the working table scheme.

8.6.4.8. Using Freedom EVO® menu or a controlling device (see operation manual), run the dosing script. Select the sample volume for NA extraction – 250 µL or 100 µL.
The dosing instrument will run automatic aliquoting of the lysis solution, internal controls, proteinase K and sorbent; preparation of negative controls, standards and introducing test samples into the DWP.

When the aliquoting is finished, there will be a signal.

8.6.5 **DWP preparation**

8.6.5.1 Using Freedom EVO® menu or a controlling device (see operation manual), select and run the DWP preparation script.
The dosing instrument will run automatic aliquoting of wash solution No. 1 (**green**), wash solution No. 2 (**yellow**), elution solution (**colorless or pink**) and lysis solution and sorbent mix.

8.6.5.2 Wait for the aliquoting and DWP preparation to finish, then install all the place into the corresponding slots of AutoPure-96 or KingFisher Flex 96. Select and run NA extraction script.

8.6.5.3 Wait for the automatic NA extraction to finish.

NA preparation is ready for PCR/RT-PCR (is in DWP No. 5 with elution solution). To store the plate with NA, use adhesive sealing film [14].

8.7 Preparation and NA extraction using package P and automatic DWP NA extraction systems

WARNING! We recommend to store cartridges with reagents at room temperature (18 °C to 25 °C) to avoid crystal formation. In case the kit has been stored at minus 18 °C and below, hold the cartridges at room temperature (18 °C to 25 °C) for at least 4 hours.

8.7.1 Prepare an automatic extraction system AutoPure-96 or KingFisher Flex 96 according to their operation manuals.

8.7.2 Prepare an empty deep-well plate (DWP) with tip comb.

8.7.3 Mark one new 96-well DWP for test samples, lysis solution and sorbent – DWP No. 1 (LB).

8.7.4 Mark 4 DWPs from the kit:

- No. 2 – with wash solution No. 1 (WB1),
- No. 3 – with wash solution No. 2 (WB2),
- No. 4 – with wash solution No. 2 (WB2),
- No. 5 – with elution solution (EB).

WARNING! The reagents are intended for up to one independent extraction procedure, given:

- 94 test samples and negative control “C-“ in 2 repeats in a run for further qualitative tests;
- 88 test samples and negative control “C-“ in 2 repeats, ST1 in 3 repeats and ST2 in 3 repeats in a run for further quantitative tests.

[14] - Not included in the kit

8.7.5 Add controls and standards according to the instructions for use for PCR/RT-PCR kits.
When using **reagent kits manufactured by "DNA-Technology"**, add[15] into each well of DWP No. 1 (LB):

- for RNA extraction – 20 µL of RNA-IC "A";
- for DNA extraction – 20 µL of DNA-IC "A";
- in case of simultaneous RNA and DNA extraction – 20 µL of RNA-IC "A" and 20 µL of DNA-IC "A".

Note. For small amounts of biomaterial, NA extraction from 100 µL of sample is possible.

8.7.6 Take off the foil from cartridge carefully, avoiding splashing of liquids.

8.7.7 Mix the tubes with sorbent and proteinase K on vortex for 3-5 seconds and spin on vortex for 1-3 seconds.

8.7.8 Add 550 µL of concentrating solution (**the solution is colorless**), 15 µL of proteinase K and 20 µL of sorbent into each well of DWP No. 1.

8.7.9 Add 250/100 µL of test samples into the corresponding wells of DWP No. 1.

8.7.10 Mix the tubes with dilution reagents and standards on vortex and spin on vortex for 1-3 seconds.

8.7.11 Add 250/100 µL of dilution reagent (depending on the sample volume) into the well for C-.

8.7.12 If quantitative test using **reagent kits manufactured by "DNA-Technology"** [16] is expected, add 20 µL of the corresponding standard and 230/80 µL of dilution reagent (depending on the sample volume) into the wells for ST1 and ST2 (3 wells for each standard).

Note: In case of quantitative test for several infections, preparation of mixed standards is allowed.
To make it, add 20 µL of ST1 from each reagent kit into each well for ST1; add 20 µL of ST2 from each reagent kit into each well for ST2. Then, reach the final volume of 250/100 µL by adding dilution reagent into the well.

Example:

1. Extraction from 250 µL:

If quantitative HCV and HBV tests are planned in one run, add 20 µL of HCV-ST1 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST1 from the **HBV Quantitative REAL-TIME PCR Kit** into ST1 tubes; add 20 µL of HCV-ST2 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST2 from the **HBV Quantitative REAL-TIME PCR Kit** into ST2 tubes. Then, add 210 µL of dilution reagent into each well.

2. Extraction from 100 µL:

If quantitative HCV and HBV tests are planned in one run, add 20 µL of HCV-ST1 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST1 from the **HBV Quantitative REAL-TIME PCR Kit** into ST1 tubes; add 20 µL of HCV-ST2 from the **HCV Quantitative REAL-TIME PCR Kit** and 20 µL of HBV-ST2 from the **HBV Quantitative REAL-TIME PCR Kit** into ST2 tubes. Then, add 60 µL of dilution reagent into each well.

8.7.13 If necessary, spin down the drops and condensate from DWP walls by centrifuging at RCF(f) 560-800 for 1-3 minutes.

Note. Use a sealed DWP with distilled water as a counterbalance.

8.7.14 Remove protective film from the plates included in the kit.

8.7.15 Install all the DWPs into the corresponding slots of Auto-Pure 96 or KingFisher Flex 96. Select and run NA extraction script.

[15] - If this is prescribed by the PCR/RT-PCR reagent kit instruction for use

[16] - If using reagent kits from other manufacturers, please refer to the instructions for use to the corresponding kits

8.7.16 Wait for the automatic NA extraction to finish.

NA preparation is ready for PCR/RT-PCR (is in DWP No. 5 with elution solution). To store the plate with NA, use adhesive sealing film.

8.8 Preparation and NA extraction using package P, DTstream dosing instrument and automatic DWP NA extraction systems

WARNING! We recommend to store cartridge with reagents at room temperature (18 °C to 25 °C) to avoid crystal formation. In case the kit has been stored at minus 18 °C and below, hold the cartridge at room temperature (18 °C to 25 °C) for at least 4 hours.

8.8.1 Prepare DTstream 15L4 or DTstream 12L4 for work according to the operation manual.

8.8.2 Prepare an automatic extraction system AutoPure-96 or KingFisher Flex 96 according to their operation manuals.

8.8.3 Prepare an empty deep-well plate (DWP) with tip comb.

8.8.4 **Sample aliquoting**

8.8.4.1 Mark one new 96-well DWP for test samples, lysis solution and sorbent – DWP No. 1 (LB).

8.8.4.2 Mark 4 DWPs from the kit:

- No. 2 – with wash solution No. 1 (WB1),
- No. 3 – with wash solution No. 2 (WB2),
- No. 4 – with wash solution No. 2 (WB2),
- No. 5 – with elution solution (EB).

WARNING! The reagents are intended for up to one independent extraction procedure, given:

- 94 test samples and negative control "C-" in 2 repeats in a run for further qualitative tests;
- 88 test samples and negative control "C-" in 2 repeats, ST1 in 3 repeats and ST2 in 3 repeats in a run for further quantitative tests.

8.8.4.3 Unpack racks with RNase and DNase free filter tips, remove the caps and install the racks onto the DTstream working table.

8.8.4.4 Install cartridge No. 1 onto the DTstream working table with the bevelled corner forward, remove the protective cover.

WARNING! Do not take off the foil from the cartridge and do not pierce it.

8.8.4.5 Install DWP No. 1 (LB) onto the DTstream working table.

8.8.4.6 Install the tubes with test samples into the rack for test samples on the DTstream working table.

8.8.4.7 Install the tubes with RNA-IC "A" or DNA-IC "A"[17], proteinase K and dilution regent into the rack for reagents. If quantitative test using **reagent kits manufactured by "DNA-Technology"**[18] is expected, install the tubes with ST1 and ST2 into the rack for reagents.

8.8.4.8 Prepare a container for waste according to the DTstream operation manual.

[17] - If this is prescribed by the PCR/RT-PCR reagent kit instruction for use.

[18] - If using reagent kits from other manufacturers, please refer to the instructions for use to the corresponding kits.

8.8.4.9 Check the readiness of DTstream:
- tip dispenser;
- the reagents, consumables and samples are installed in accordance with the working table scheme.

8.8.4.10 Using DTstream menu or a controlling device (see operation manual), run the dosing script. Select the sample volume for NA extraction – 250 µL or 100 µL. The dosing instrument will run automatic aliquoting of the lysis solution, internal controls, proteinase K and sorbent; preparation of negative controls, standards and introducing test samples into the DWP.

8.8.4.11 Wait for the aliquoting to finish.

8.8.4.12 Prepare AutoPure-96 or KingFisher Flex 96 by installing DWP with tip comb and DWP No. 1 (LB) into the corresponding slots according to the operation manual.

8.8.4.13 If necessary, spin down the drops and condensate from DWP walls by centrifuging at RCF(f) 560-800 for 1-3 minutes.

N o t e . Use a sealed DWP with distilled water (750 µL in each well) as a counterbalance.

8.8.4.14 Remove protective film from the plates included in the kit.

8.8.5 Install all the DWPs into the corresponding slots of Auto-Pure 96 or KingFisher Flex 96. Select and run NA extraction script.

8.8.6 Wait for the automatic NA extraction to finish.

NA preparation is ready for PCR/RT-PCR (is in DWP No. 5 with elution solution). To store the plate with NA, use adhesive sealing film.

8.9 Preparation and NA extraction using package P, Freedom EVO® (TECAN) dosing instrument and automatic DWP NA extraction systems

WARNING! We recommend to store cartridges with reagents at room temperature (18 °C to 25 °C) to avoid crystal formation. In case the kit has been stored at minus 18 °C and below, hold the cartridges at room temperature (18 °C to 25 °C) for at least 4 hours.

8.9.1 Prepare Freedom EVO® for work according to the operation manual.

8.9.2 Prepare an automatic extraction system AutoPure-96 or KingFisher Flex 96 according to their operation manuals.

8.9.3 Prepare an empty deep-well plate (DWP) with tip comb.

8.9.4 **Sample aliquoting**

8.9.4.1 Mark one new 96-well DWP for test samples, lysis solution and sorbent – DWP No. 1 (LB).

8.9.4.2 Mark 4 DWPs from the kit:
- No. 2 – with wash solution No. 1 (WB1),
- No. 3 – with wash solution No. 2 (WB2),
- No. 4 – with wash solution No. 2 (WB2),
- No. 5 – with elution solution (EB).

WARNING! The reagents are intended for up to one independent extraction procedure, given:
- 94 test samples and negative control “C-“ in 2 repeats in a run for further qualitative tests;
- 88 test samples and negative control “C-“ in 2 repeats, ST1 in 3 repeats and ST2 in 3 repeats in a run for further quantitative tests.

8.9.4.3 Unpack racks with RNase and DNase free filter tips, remove the caps and install the racks onto the Freedom EVO® working table.

8.9.4.4 Install cartridge onto the Freedom EVO® working table with the bevelled corner forward, remove the protective cover.

WARNING! Do not take off the foil from the cartridges and do not pierce it.

8.9.4.5 Install an empty DWP No. 1 (LB) onto the Freedom EVO® working table.

8.9.4.6 Install the tubes with test samples into the rack for test samples on the Freedom EVO® working table.

8.9.4.7 Install the tubes with RNA-IC "A" or DNA-IC "A"[19], proteinase K and dilution reagent into the rack for reagents. If quantitative test using **reagent kits manufactured by "DNA-Technology"**[20] is expected, install the tubes with ST1 and ST2 into the rack for reagents.

8.9.4.8 Check the readiness of Freedom EVO®:
- tip dispenser;
- the reagents, consumables and samples are installed in accordance with the working table scheme.

8.9.4.9 Using Freedom EVO® menu or a controlling device (see operation manual), run the dosing script. Select the sample volume for NA extraction – 250 µL or 100 µL.
The dosing instrument will run automatic aliquoting of the lysis solution, internal controls, proteinase K and sorbent; preparation of negative controls, standards and introducing test samples into the DWP.

8.9.4.10 Wait for the aliquoting to finish.

8.9.4.11 If necessary, spin down the drops and condensate from DWP walls by centrifuging at RCF(f) 560-800 for 1-3 minutes.

N o t e . Use a sealed DWP with distilled water as a counterbalance.

8.9.4.12 Remove protective film from the plates included in the kit.

8.9.5 Install all the DWPs into the corresponding slots of Auto-Pure 96 or KingFisher Flex 96. Select and run NA extraction script.

8.9.6 Wait for the automatic NA extraction to finish.

NA preparation is ready for PCR/RT-PCR (is in DWP No. 5 with elution solution). To store the plate with NA, use adhesive sealing film.

8.10 NA preparation storage and use

8.10.1. If NA preparation is intended for RT or RT-PCR (RNA test), NA preparation storage is allowed at 2 °C – 8 °C for up to 2 hours.

8.10.2. If NA preparation is intended for PCR (DNA test), NA preparation storage is allowed at minus 22 °C – minus 18 °C for up to 1 month or at minus 72 °C – minus 68 °C for up to 1 year without thawing prior to the test.

WARNING!

1. For package N: To ensure safe storage of NA preparation (for DNA test) transfer supernatant into the new marked tubes.
2. Only one NA preparation thawing is allowed!

8.10.3. If NA preparations have been stored at minus 22 °C – minus 18 °C or at minus 72 °C – minus 68 °C, thaw NA preparations, negative control and standards at room temperature (18 °C – 25 °C) before use.

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[19] - If this is prescribed by the PCR/RT-PCR reagent kit instruction for use.

[20] - If using reagent kits from other manufacturers, please refer to the instructions for use to the corresponding kits.

WARNING! Only one thawing is allowed for NA preparation!

8.10.4. Before using NA preparation for PCR/RT-PCR after storage:

- packages A, P, N (extraction in DWPs):

Centrifuge DWP with NA preparation, negative control and standards at RCF(g) 100 for 30 seconds to spin down the condensate. Take off the adhesive film.

- package N (extraction in 1.5-2.0 mL tubes):

Place the tubes with NA preparation, negative control and standards into the magnetic rack.

If after extraction supernatant containing the extracted NA was transferred into new tubes, shake the tubes with NA preparation, negative control and standards on vortex for 3-5 seconds and spin on vortex for 1-3 seconds.

NA preparation is ready for PCR/RT-PCR.

9. SPECIFICATIONS

a. The **minimal amount of biomaterial** for nucleic acids extraction is 100 µL.

The recommended amount of biomaterial to increase sensitivity of the test is 250 µL.

b. **Functional parameters of the kit:**

NA concentration in 100 µL of eluate is 0.06 – 0.30 ng/µL. Consider that the kit is designed for NA extraction from plasma/serum free from blood cells to detect viral RNA/DNA.

WARNING! The eluate contains nonionic detergent absorbing in the UV region of spectrum (λmax = 275±5 nm) and hindering the quantitative detection of protein admixture.

c. **Effectiveness of the reagent kit**

The effectiveness of the reagent kit was determined in clinical trials using additional PCR reagent kits for biomaterial samples test:

Biomaterial	Test samples, pcs	Analyte	PREP-MB-LITE effectiveness
Blood plasma	50	HBV	100 % (P_{true} = 95.50)
	50	HCV	100 % (P_{true} = 95.50)
Blood serum	50	HBV	100 % (P_{true} = 95.50)
	50	HCV	100 % (P_{true} = 95.50)

d. Medical devices compatible with the **PREP-MB-LITE viral DNA/RNA Extraction Kit**:

The **PREP-MB-LITE viral DNA/RNA Extraction Kit** for viral nucleic acid extraction from blood plasma and blood serum can be used together with the PCR/RT-PCR reagent kits.

The **PREP-MB-LITE viral DNA/RNA Extraction Kit** for viral nucleic acid extraction from blood plasma and blood serum is validated with the following PCR/RT-PCR reagent kits:

- **HCV Quantitative REAL-TIME PCR Kit** ("DNA-Technology R&P", LLC, Russia);
- **HCV REAL-TIME PCR Kit** ("DNA-Technology R&P", LLC, Russia);
- **HBV Quantitative REAL-TIME PCR Kit** ("DNA-Technology R&P", LLC, Russia);
- **HBV REAL-TIME PCR Kit** ("DNA-Technology R&P", LLC, Russia).

10. QUALITY CONTROL

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

- observation of quality management in manufacturing of 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-MB-LITE viral DNA/RNA Extraction Kit**:

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

Manufacturer: “DNA-Technology Research&Production”, LLC,

142281, Russia, Moscow region,

Protvino, Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Seller: "DNA-Technology" LLC,

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int. ter. Municipal District Chertanovo Severnoye,

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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		

REF P-136-A/9ER
P-136-N/9ER
P-136-P/9ER
P-137-P/9ER

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