RIBO-prep nucleic acid extraction kit

CE IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		GHS02: Flame
	Manufacturer		GHS05: Corrosion
	Date of manufacture		GHS07: Exclamation mark
EC REP	Authorized representative in the European Community		

1. INTENDED USE

RIBO-prep nucleic acid extraction kit is intended for extraction and purification of total RNA/DNA from clinical material (peripheral blood plasma, cerebrospinal and amniotic fluid, nasal and oropharyngeal swabs, and saliva) for subsequent analysis with reverse transcription and polymerase chain reaction.

Indications and contra-indications for use of the reagent kit

RNA/DNA extraction is used in preanalytical stage of in vitro diagnostics by nucleic acid amplification techniques (NAT).

2. PRINCIPLE OF NUCLEIC ACID EXTRACTION

Test samples are treated by **Solution for Lysis** to destruct cell membranes, viral envelopes and other biopolymer complexes and release nucleic acids and cellular components. The dissolved RNA/DNA precipitates after addition of the **Solution for Precipitation** and centrifugation, while the other components of the lysed clinical material remain in the solution and removed with subsequent washes. The final stage of extraction is dissolution of the pellet in elution buffer, the purified RNA/DNA is transferred into the solution.

The result of the specified procedure is the purified RNA/DNA and the absence of PCR inhibitors which ensures the high analytical sensitivity of the PCR analysis.

RNA/DNA extraction is a preanalytical phase in the clinical laboratory diagnostics.

3. CONTENT

RIBO-prep nucleic acid extraction kit is produced in 1 form:

variant 100, REF K2-9-Et-100-CE.

Variant 100 includes:

Reagent	Description	Volume, ml	Quantity
Solution for Lysis	clear liquid from colorless to blue grey colour[1]	30	1 vial
Solution for Precipitation	colorless clear liquid	40	1 vial
Washing Solution 3	colorless clear liquid	50	1 vial
Washing Solution 4	colorless clear liquid	20	1 vial
RNA-buffer	colorless clear liquid	1.2	8 tubes

Variant 100 is intended for 100 RNA/DNA extraction, including controls.

4. ADDITIONAL REQUIREMENTS

- Sterile RNase-free pipette tips with aerosol filters (up to 10 µl, 200 µl and 1000 µl).
- Tube racks.
- Vortex mixer.
- Thermostat with working temperature for 25-100 °C.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- Vacuum aspirator with flask for removing supernatant.
- PCR box or Biological cabinet.
- Disposable 1.5-ml polypropylene sterile tubes.
- Pipettes (adjustable).
- Refrigerator for 2-8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette filter tips and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the kit if the internal packaging was damaged or its appearance was changed.
- Do not use the kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5% sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The kit is intended for analysis of specified number of samples (see the section “Content”).
- The kit is ready for use in accordance with the Instruction Manual. Use the kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area where the previous step was performed.

NOTE: **Solution for Lysis** has an unpleasant smell. Work with this solution should be performed in a biological cabinet.

Solution for Lysis Danger	Contains substance: guanidine thiocyanate. H302: Harmful if swallowed. H312: Harmful in contact with skin. H314: Causes severe skin burns and eye damage. H332: Harmful if inhaled. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P260: Do not breathe vapours. P264: Wash your hands thoroughly after handling. P273: Avoid release to the environment. P302+P352: IF ON SKIN: Wash with plenty of water. P501: Dispose of contents in accordance with national regulation.
Washing Solution 3 Warning	Contains substance: isopropyl alcohol H226: Flammable liquid and vapour. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P261: Avoid breathing vapours. P264: Wash your hand thoroughly after handling. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. P403+P233: Store in a well ventilated place. Keep container tightly closed. P501: Dispose of contents in accordance with national regulation.
Solution for Precipitation, Washing Solution 4 Danger	Isopropanol EC No 200-661-7 CAS No 67-63-0 H225: Highly flammable liquid and vapour. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P261: Avoid breathing vapours. P264: Wash your hand thoroughly after handling. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. P403+P233: Store in a well ventilated place. Keep container tightly closed. P501: Dispose of contents in accordance with national regulation.

[1] If Solution for Lysis is stored at 2-8 °C, a crystalline precipitate may form.

6. SAMPLING AND HANDLING

NOTE: Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

RIBO-prep nucleic acid extraction kit is recommended for **RNA and DNA** extraction and purification from:

- *blood plasma*
- *cerebrospinal fluid (liquor)*
- *amniotic fluid*
- *nasal swabs*
- *oropharyngeal swabs*
- *saliva*

Interfering substances and limitations of using test material samples

The information about potential interfering substances and limitations of using test material samples is specified in the Instruction Manual of the PCR kit.

Potential interfering substances

Endogenous and exogenous substances that may be present in the clinical material (blood plasma, liquor, amniotic fluid, nasal and oropharyngeal swabs, saliva) used for the study were selected to assess potential interference (see Table 1).

Model samples of various clinical material without adding and with the addition of potentially interfering substances were tested. The concentration of each potentially interfering substance is listed in Table 1.

Table 1

Test material	Type of potential interferent	Potential interferent	Tested concentration in a sample	Interference presence
Blood plasma	Endogenous substances	Hemoglobin	5 g/l (upper limit of normal 1 g/l)	Not detected
		Triglycerides	37 mmol/l (upper limit of normal 3.7 mmol/l)	Not detected
		Bilirubin	210 µmol/l (upper limit of normal 21 µmol/l)	Not detected
		Protein	120 g/l (upper limit of normal 85 g/l)	Not detected
Liquor	Exogenous substances	Whole blood	Up to 4 % volume/volume	Not detected
		Leukocytes	500 cells/mm^3	Not detected
Nasal and oropharyngeal swabs / saliva	Endogenous substances	Mucin	5 %	Not detected
	Exogenous substances	Lugol's solution with glycerin	0.5 %	Not detected
		Chlorhexidine bigluconate aqueous solution	2.5 %	Not detected
Amniotic fluid	Exogenous substances	Cefazolin sodium salt	64 µg/ml	Not detected

7. WORKING CONDITIONS

RIBO-prep nucleic acid extraction kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. RNA and DNA Extraction

1. Warm **Solution for Lysis** (if stored at 2-8 °C) at the temperature **65 °C** until crystals disappear.
2. Take required number of 1.5 ml disposable tubes with tightly closable caps including one tube for **Negative Control of Extraction** (**C-**) and one tube for **Positive Control of Extraction** (**PCE)**. Add **10 µl** of **Internal Control** (if it is provided for analysis of this infectious agent) to each tube and then add **300 µl** of **Solution for Lysis**. Mark the tubes.
3. Add **100 µ**l of prepared samples to the tubes with **Solution for Lysis** and **Internal Control** (if used) using pipette tips with aerosol filters. Add **100 µl** of **Negative Control** to the tube labeled **C–**. Add **90 µl** of **Negative Control** and **10 µ**l of **Positive Control** (if it is provided for analysis) to the tube labeled **PCE.**
4. Mix the contents of the tubes thoroughly by vortexing, then centrifuge tubes for 5 s to be sure there are no drops on the cap, and incubate them at **65 °C** for **5 min**.
5. Add **400 µl** of **Solution for Precipitation** and mix by vortexing.
6. Centrifuge all tubes for **5 min** at **13,000 rpm**.
7. Carefully remove the supernatant without disturbing the pellet using a vacuum aspirator and 200-µl tips. Use a new tip for each tube.
8. Add **500 µl of Washing Solution 3** to each tube, tightly close the tubes and turn them carefully upside down 3–5 times to wash the pellet. This procedure can be performed simultaneously for all the tubes: cover the tubes placed in a rack with a lid or another rack, press them, and turn the rack.
9. Centrifuge all the tubes at **13,000 rpm** for **1–2 min**.
10. Carefully remove the supernatant without disturbing the pellet using a vacuum aspirator and 10-µl tips. Use a new tip for each tube.
11. Add **200 µl of Washing Solution 4** to each tube, tightly close the tubes and turn them carefully upside down and back 3–5 times to wash the pellet.
12. Centrifuge all tubes at **13,000 rpm** for **1–2 min**.
13. Carefully remove the supernatant without disturbing the pellet using a vacuum aspirator and 10-µl tips. Use a new tip for each tube.
14. Incubate all tubes with open caps at **65 °C for 5 min** (to dry the pellet).
15. Add **50 µl** of **RNA buffer** into each tube. Mix the tubes by vortex. Then incubate them at **65 °C** for **5 min** occasionally stirring by vortex. Elution volume can be increased up to 90 µl.
16. Centrifuge the tubes at **13,000 rpm** for **1 min**. The supernatant contains purified RNA and DNA. Samples are ready for reverse transcription and PCR.

The purified RNA/DNA can be stored:

- at 2-8 °C for 24 h;
- at the temperature not more than minus 16 °C for 1 year.

8.2. Amplification

It's recommended to use AmpliSens® PCR kits and REVERTA-L reverse transcription reagents kit.

NOTE: Carry out the amplification according to the manufacturer instruction.

9. TROUBLESHOOTING

These troubleshooting rules may be helpful in explaining any questions that may arise.

False negatives with extraction product:

- Degradation of the nucleic acid contained in the sample. It is necessary to use a new sample. Store samples under appropriate conditions.
- Loss of nucleic acid pellet. Carefully discard the washing solution trying not to disturb the nucleic acid pellet.
- Degradation of the extracted nucleic acid. It is necessary to use DNase- and RNase-free plastic.

False positives with extraction product:

- Contamination during sample extraction. It is necessary to open one test tube at a time. Avoid spilling the contents of the test tube. Always change tips.
- Contamination of the reagents prepared for the step. It is necessary to repeat the test.
- Contamination of the extraction zone by amplicons. It is necessary to clean surfaces and instruments using aqueous detergents, wash lab coats, and replace test tubes and tips in use. Use different laboratory coats in different areas.

If you have any questions or encounter problems, please contact our Authorized Representative in the European Community.

10. TRANSPORTATION

RIBO-prep nucleic acid extraction kit should be transported at 2–25 ºC for no longer than 5 days.

11. STABILITY AND STORAGE

All components of **RIBO-prep** nucleic acid extraction kit are to be stored at 2-8ºC, when not in use. All components of **RIBO-prep** nucleic acid extraction kit are to be stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

12. REFERENCES

1. Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being.

13. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Total Quality Management System, each lot of **RIBO-prep** nucleic acid extraction kit has been tested against predetermined specifications to ensure consistent product quality.

Please contact our Authorized representative in the European Community if side effects, undesirable reactions, facts and circumstances that pose a threat to the life and health of citizens and medical workers are identified during the use of the reagent kit.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
10.12.10	Cover page	The phrase "For Professional Use Only" was added
	Content	New sections "Working Conditions" and "Transportation" were added
		The "Explanation of Symbols" section was renamed to "Key to Symbols Used
	Stability and Storage	The information about the shelf life of open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
	Content	The color of Solution for Lysis was changed into blue
		The volume of Washing Solution 3 was changed into 25 ml (for variant 50)
		The reference «If Solution for Lysis is stored at 2-8 °C, a crystalline precipitate may form» was added
04.07.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
06.09.11 RT	8. Protocol 8.1. RNA/ DNA extraction	Procedure of extraction was corrected (heating at 65 °C for 5 min was added in Section 8.1, article 4).
12.07.12 BM	5. General precautions	Information about an unpleasant smell of Solution for Lysis and the necessity to work in a biological cabinet was added
31.03.15 ME	5. General precautions, 14. Key to symbols used	Information about hazards was corrected
10.05.17 PM	Through the text	Correction according to the template
	5. General precautions, 14. Key to symbols used	Information about hazards was rewritten according to the Regulation 1272/2008/EC.
27.03.18 PM	3. Content	The color of the reagent was specified
09.04.20 KK	Through the text	The text formatting was changed
	Footer	The phrase "Not for use in the Russian Federation" was added
21.10.20 KK	Footer, 3. Content	The information about variant 50, REF K2-9-Et-50-CE was deleted
11.03.21 VA	—	The name, address and contact information for Authorized representative in the European Community was changed
12.08.21 EM	Interfering substances and limitations of using test material samples	The sections were added
	Principle of nucleic acid extraction	
	Through the text	Corrections according to the template
31.05.22 KK	1. Intended use	"Indications and contra-indications for use of the reagent kit" subsection was added
	5. General precautions	The phrase "for single use" was deleted
	13. Quality control	The Authorized representative in the European Community was specified for the contact in case of undesirable effects when using the reagent kit

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