

RIBO-zol-B nucleic acid extraction kit



For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

	Catalogue number		Contains sufficient for <n> tests
	Batch code		Use-by Date
	In vitro diagnostic medical device		Consult instructions for use
	Version		GHS07: Exclamation mark
	Temperature limit		GHS06: Skull and crossbones
	Manufacturer		GHS08: Health hazard
	Date of manufacture		GHS05: Corrosion
	Authorized representative in the European Community		GHS02: Flame
	Caution		

1. INTENDED USE

RIBO-zol-B nucleic acid extraction kit is intended for extraction of total RNA from clinical materials for further analysis by using reverse transcription and polymerase chain reaction method.

Indications and contra-indications for use of the reagent kit

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

2. PRINCIPLE AND PROCEDURE

RIBO-zol-B nucleic acid extraction kit is the reagents kit for rapid and efficient manual extraction and purification of RNA from various clinical materials. Solution D contains chaotropic agent (guanidine thiocyanate) that lyses cells and denaturates cell proteins. RNA extracted from clinical samples may be used for PCR diagnostic tests.

3. CONTENT

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

variant 100, K2-3-100-CE.

Variant 100 include:

Reagent	Description	Volume, ml	Quantity
Solution D	colorless clear liquid	40	1 vial
Solution E	colorless clear liquid	1.5	2 tubes
Solution A	yellow clear liquid	30	1 vial
Solution B	colorless clear liquid	10	1 vial
Solution C	colorless clear liquid	40	1 vial
Washing Solution 3	colorless clear liquid	100	1 vial
RNA-eluent	colorless clear liquid	0.5	10 tubes

Variant 100 is intended for RNA extraction from 100 samples, including controls.

4. ADDITIONAL REQUIREMENTS




- Homogenizer (separate for each sample) – porcelain mortar with pestle, and kit of sterile instruments.
- Vacuum aspirator with flask for removing supernatant.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl and up to 1000 µl).
- Sterile RNase-free pipette tips (up to 200 µl).
- Vortex mixer.
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RCF max. 16,000 x g).
- PCR box or Biological cabinet.
- Thermostat for tubes with controlled temperature for 25-100 °C.
- Tube racks.
- Disposable 1.5 ml polypropylene sterile tubes.
- Refrigerator for 2–8 °C
- Deep-freezer at the temperature from minus 24 to minus 16 °C
- Reservoir for used tips.


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 samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- 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.

 Solution A Danger	<p>Phenol EC No 203-632-7 CAS No 108-95-2</p> <p>H301: Toxic if swallowed. H311: Toxic in contact with skin. H314: Causes severe skin burns and eye damage. H331: Toxic if inhaled. H341: Suspected of causing genetic defects. H373: May cause damage to organs through prolonged or repeated exposure. H411: Toxic to aquatic life with long lasting effects.</p> <p>P201: Obtain special instructions before use. P260: Do not breathe vapours. P264: Wash your hands thoroughly after handling. P303+ P361+ P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P405: Store locked up. P501: Dispose of contents in accordance with national regulation.</p>
 Solution D Danger	<p>Contains substance: guanidine thiocyanate</p> <p>H302: Harmful if swallowed. H312: Harmful in contact with skin. H314: Causes severe skin burns and eye damage H317: May cause an allergic skin reaction H332: Harmful if inhaled. H412: Harmful to aquatic life with long lasting effects.</p> <p>EUH032: Contact with acids liberates very toxic gas.</p> <p>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.</p>
 Solution B Danger	<p>Contains substance: chloroform</p> <p>H302: Harmful if swallowed. H315: Causes skin irritation. H319: Causes serious eye irritation. H331: Toxic if inhaled. H336: May cause drowsiness or dizziness. H351: Suspected of causing cancer. H361d: Suspected of damaging the unborn child. H372: Causes damage to organs through prolonged or repeated exposure.</p> <p>P261: Avoid breathing dust/fume/ gas/mist/vapours/spray. P280: Wear protective gloves/protective clothing/eye protection/face protection. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. P311: Call a POISON CENTER or a doctor. P501: Dispose of contents in accordance with national regulation.</p>

 <p>Solution C</p> <p>Danger</p>	<p>Isopropanol EC No 200-661-7 CAS No 67-63-0</p> <p>H225: Highly flammable liquid and vapour. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness</p> <p>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.</p> <p>Contains substance: acetic acid</p>
 <p>Solution E</p> <p>Danger</p>	<p>H226: Flammable liquid and vapour H314: Causes severe skin burns and eye damage</p> <p>P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P280: Wear protective gloves/protective clothing/eye protection/face protection. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. P310: Immediately call a poison center or doctor. P405: Store locked up. P501: Dispose of contents in accordance with national regulation.</p> <p>Contains substance: isopropyl alcohol</p>
 <p>Washing Solution 3</p> <p>Warning</p>	<p>H226: Flammable liquid and vapour. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness</p> <p>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.</p>

 Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [2]. It is recommended that this handbook is read before starting work.

RIBO-zol-B nucleic acid extraction kit is recommended for RNA extraction and purification from 30 mg (or 30 µl) of clinical materials.

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.

7. WORKING CONDITIONS

RIBO-zol-B nucleic acid extraction kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA Extraction

- Transfer **30 mg** (or 30 µl) of **biopsy material** (brain, liver, spleen or lymph nodes tissues) into porcelain mortar and homogenize it using the teflon pestle. Add **300 µl** of **Solution D** and grind by pestle until the solution become nonviscous.
- Transfer the homogenate into disposable tube with tightly closed lid. Centrifuge for removing drops from the lids of the tubes.
- Add **30 µl** of **Solution E**. Mix on vortex.
- Add **300 µl** of **Solution A**. Mix on vortex.
- Add **100 µl** of **Solution B**. Mix on vortex for 1-2 min (solution should become milky-white).
- Place the tubes into ice bath (2-4 °C) for 10 min.
- Centrifuge the tubes at 14,000-16,000 rpm for 10 min. Solution should separate into 2 phases: bottom phase, which contains proteins and DNA, and top phase, which contains RNA.
- Carefully remove top phase (about 0.3 ml), using tips with aerosol filter, and transfer it in a new tube.
- Add **300 µl** of **Solution C**. Mix on vortex and incubate in a deep-freezer at the temperature not more than minus 16 °C for 1 hour.
- Centrifuge the tubes at 14,000-16,000 rpm for 10 min. Remove the supernatant (do not disturb the sediment).
- Dilute the sediment in **100 µl** of **Solution D**, add **100 µl** of **Solution C**. Mix on vortex. Incubate in a deep-freezer at the temperature not more than minus 16 °C for 1 hour.
- Centrifuge the tubes at 14,000-16,000 rpm for 10 min. Remove the supernatant (do not disturb the sediment).
- Wash the sediment in **800 µl** of **Washing Solution 3**, cooled at 2-8 °C. Mix on vortex. Centrifuge the tubes at 14,000-16,000 rpm for 10 min. Remove the supernatant (do not disturb the sediment).
- Repeat p. 12 after addition of **200 µl** of cooled **Washing Solution 3**.
- Place the tubes into the thermostat at 60 °C for 5 min for sediment pre-drying (tube lids are to be opened).
- Add **30 µl** of **RNA-eluent** into the tubes.
RNA solution should be stored at the temperature not more than minus 68 °C.
50 µl of high-purity RNA can be extracted from 30 mg of brain, liver, spleen or lymph nodes tissues.

8.2. Amplification

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

NOTE: Please carry out the amplification according to the manufacturer's instructions.

9. TROUBLESHOOTING

False negatives with extraction product:

- Degradation of the nucleic acid contained in the sample. It's necessary to use a new sample. Store samples under appropriate conditions.
- Loss of nucleic acid residue. Carefully draw off the washing solution and try not to remove the nucleic acid residue.
- Degradation of the extracted nucleic acid. It's necessary to use DNase- and RNase-free plastic.

False positives from extracted product:

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

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

10. TRANSPORTATION

RIBO-zol-B nucleic acid extraction kit should be transported at 2–8 °C for no longer than 5 days.

11. STABILITY AND STORAGE

All components of the **RIBO-zol-B** nucleic acid extraction kit are to be stored at 2-8 °C (except for RNA-eluent), when not in use. All components of the **RIBO-zol-B** nucleic acid extraction kit are to be stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: RNA-eluent is to be stored at temperature from minus 24 to minus 16 °C when not in use.

12. REFERENCES

- Chomczynski P. and Sacchi N. Anal.Biochem 1987, V.162, P.156-159.
- 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 Quality Management System, each lot of the **RIBO-zol-B** 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
27.12.10 KM	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
04.07.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
20.06.13 FN	General precautions	The Corrosive Warning symbol was added
	Key to Symbols Used	
31.03.15 ME	5. General precautions, 14. Key to symbols used	Information about hazards was corrected
22.09.17 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.
08.04.20 MM	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 K2-3-50-CE was deleted
12.03.21 VA	—	The name, address and contact information for Authorized representative in the European Community was changed
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
	6. Sampling and handling	"Interfering substances and limitations of using test material samples" subsection was added
	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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