CE

IVD

For Professional Use Only

RIBO-zol-B

nucleic acid extraction kit

Instruction Manual

AmpliSens®

EC REP

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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.

2. PRINCIPLE OF NUCLEIC ACID EXTRACTION

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 2 forms:

RIBO-zol-B nucleic acid extraction kit variant 50, REF K2-3-50-CE.

RIBO-zol-B nucleic acid extraction kit variant 100, REF K2-3-100-CE.

RIBO-zol-B nucleic acid extraction kit variant 50 or 100 includes:

Reagent	***Description***	***variant 50***		***variant 100***	
		Volume (ml)	***Amount***	***Volume (ml)***	***Amount***
Solution D	colorless clear liquid	20	1 vial	40	1 vial
Solution E	colorless clear liquid	1.5	1 tube	1.5	2 tubes
Solution A	yellow clear liquid	15	1 vial	30	1 vial
Solution B	colorless clear liquid	5.0	1 tube	10.0	1 vial
Solution C	colorless clear liquid	20	1 vial	40	1 vial
Washing Solution 3	colorless clear liquid	50	1 vial	100	1 vial
RNA-eluent	colorless clear liquid	0.5	5 tubes	0.5	10 tubes

RIBO-zol-B nucleic acid extraction kit variant 50 is intended for RNA extraction from 50 samples, including controls.

RIBO-zol-B nucleic acid extraction kit variant 100 is intended for RNA extraction from 100 samples, including controls.

4. ADDITIONAL REQUIREMENTS

- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol barriers (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.
- Vacuum aspirator with flask for removing supernatant.
- Tube racks.
- 1.5 ml polypropylene sterile tubes (tightly closed or screwed).
- Refrigerator for 2–8 °C with deep-freezer for ≤ –16 °C.
- Waste bin for used tips.
- Permanent pen for labeling.
- Thermostat for tubes with capable of incubating at 25-100 °C.
- Homogenizer (separate for each sample) – porcelain mortar with pestle, and kit of sterile instruments.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile RNase-free pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, 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 a kit after its expiry date.
- Dispose of all samples and unused reagents in compliance with local authorities requirements.
- 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 other suitable disinfectant.
- Avoid contact with the skin, eyes and mucose membranes. If skin, eyes and mucose membranes contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one directional; it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.

 Xn	**Solution D**	Contains guanidine thiocyanate. Guanidine thiocyanate is harmful if inhaled, or comes in contact with skin or if swallowed. Contact with acid releases toxic gas. Harmful (Xn). Risk and safety phrases:* R20/21/22-32, S13-26-36-46
 T	**Solution A**	Contains phenol: toxic (T), corrosive (C) Risk and safety phrases:* R23/24/25-34-48/20/21/22-68 S24/25-26-28-36/37/39-45
 Xn	**Solution B**	Contains chlorophorm: harmful (Xn). Risk and safety phrases: * R22-38-40-48/20/22, S36/37
 F	**Solution C**	Contains isopropanol: Highly flammable (F), Irritant (Xi) Risk and safety phrases: * R11-36-67, S7-16-24/25-26
 F	**Washing Solution 3**	Contains ethanol: flammable. Risk phrase:* R10

*R10: Flammable;
R11: Highly flammable;
R20: Harmful if inhalation;
R22: Harmful if swallowed;
R32: Contact with acids liberates very toxic gas;
R34: Causes burns;
R36: Irritating to eyes;
R37: Irritating to the respiratory system;
R38: Irritating to the skin;
R40: Limited evidence of a carcinogenic effect;

R66: Repeated exposure can cause skin dryness or cracking;
R67: Vapours may cause drowsiness and dizziness;
R68: Possible risk of irreversible effect;
R20/21/22: Harmful by inhalation, in contact with skin and if swallowed;
R23/24/25: Toxic by inhalation, in contact with skin and if swallowed;
R36/37/38: Irritating to eyes, respiratory system and skin;
R42/43: May cause sensitization by inhalation and skin contact;
R48/20/21/22:Harmful: danger of serious damage to health by prolonged exposure through inhalation and in contact with skin and if swallowed;
R48/20/22: Harmful: danger of serious damage to health by prolonged exposure through inhalation and if swallowed
S 7: Keep container tightly closed;
S13: Keep away from food, drink and animal feeding stuffs;
S16: Keep away from sources of ignition - No smoking;
S22: Do not breathe dust;
S23: Do not breathe spray;
S24: Avoid contact with skin;
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice;
S28: After contact with skin, wash immediately with plenty of water;
S36: Wear suitable protective clothing;
S45: In case of accident or if you feel unwell, seek medical advice immediately (show label where possible);
S46: If swallowed, seek medical advice immediately and show the container or label;
S24/25: Avoid contact with skin and eyes;
S36/37: Wear suitable protective clothing and gloves;
S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection.

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.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. RNA Extraction

1. Transfer **30 mg** (or 30 µl) of **biopsy material** (brain, liver, spleen or lymph nodes tissues) into porcelain mortar and homogenize it by the teflon pestle. Add **300 µl** of **Solution D** and grind by pestle until the solution become nonviscous.
2. Transfer the homogenate into disposable tube with tightly closed lid. Centrifuge for removing drops from the lids of the tubes.
3. Add **30 µl** of **Solution E**. Mix on vortex.
4. Add **300 µl** of **Solution A**. Mix on vortex.

5. Add **100 µl** of **Solution B**. Mix on vortex for 1-2 min (solution should become milky).
6. Place the tubes into ice bath (temperature between 2 and 4 °C) for 10 min.
7. 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.
8. Carefully remove top phase (about 0.3 ml), using tips with aerosol barrier, and transfer it in a new tube.
9. Add **300 µl** of **Solution C**. Mix on vortex and incubate in a deep-freezer at ≤ –16 °C for 1 hour.
10. Centrifuge the tubes at 14,000-16,000 rpm for 10 min. Remove the supernatant (do not disturb the sediment).
11. Dilute the sediment in **100 µl** of **Solution D**, add **100 µl** of **Solution C**. Mix on vortex. Incubate in a deep-freezer at ≤ –16 °C. for 1 hour.
12. Centrifuge the tubes at 14,000-16,000 rpm for 10 min. Remove the supernatant (do not disturb the sediment).
13. 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).
14. Repeat p.12 after addition of **200 µl** of cooled **Washing Solution 3**.
15. Place the tubes into the thermostat at 60 °C for 5 min for sediment predrying (tube lids are to be opened).
16. Add **30 µl** of **RNA-eluent** into the tubes.

RNA solution is to be stored at the temperature not more than 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.

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. Use a new sample, store samples appropriately.

- Loss of nucleic acid deposit. Carefully draw off the wash solution and try not to remove the nucleic acid deposit.
- Degradation of the extracted nucleic acid. Plastic free from DNAses and RNAses should be used. Use a new aliquot of kit's component.

False positives from extracted product:

- Contamination during sample extraction. One test tube at a time should be opened. 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 Amplification 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 ºC.

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.

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

12. REFERENCES

1. Chomczynski P. and Sacchi N. Anal.Biochem 1987, V.162, P.156-159.
2. Manual "Sampling, transportation and storage of clinical material for PCR diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology", Moscow, 2008.

13. QUALITY CONTROL

In accordance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485 – certified Total Quality Management System, each lot of **RIBO-zol-B** nucleic acid extraction kit is tested against predetermined specifications to ensure consistent product quality.

14. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Harmful
	Manufacturer		Toxic
	Date of manufacture		Flammable
EC REP	Authorised representative in the European Community		

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”