



For Professional Use Only

RIBO-zol-C

nucleic acid extraction kit

Instruction Manual

AmpliSens[®]



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TABLE OF CONTENTS

1. INTENDED USE	3
2. PRINCIPLE OF NUCLEIC ACID EXTRACTION	3
3. CONTENT	3
4. ADDITIONAL REQUIREMENTS	3
5. GENERAL PRECAUTIONS.....	4
6. SAMPLING AND HANDLING	5
7. WORKING CONDITIONS.....	6
8. PROTOCOL	6
9. TROUBLESHOOTING.....	7
10. TRANSPORTATION.....	7
11. STABILITY AND STORAGE.....	7
12. REFERENCES	7
13. QUALITY CONTROL.....	7
14. KEY TO SYMBOLS USED	8

1. INTENDED USE

RIBO-zol-C nucleic acid extraction kit is intended for the first stage of extraction of total RNA from clinical biological materials. Following purification and concentration of RNA performed by sorption or precipitation methods are required.

2. PRINCIPLE OF NUCLEIC ACID EXTRACTION

RIBO-zol-C nucleic acid extraction kit is the reagents kit for rapid and efficient first stage of RNA manual extraction from various biological materials. Solution D contains chaotropic agent (guanidine thiocyanate) that lyses cells and denaturates cell proteins. Hydrous phase, obtained after addition of Solution A, Solution B and Solution E, contains RNA. After further purification and concentration it can be used in PCR diagnostic tests.

3. CONTENT

RIBO-zol-C nucleic acid extraction kit is produced in 2 forms:

RIBO-zol-C nucleic acid extraction kit variant 50, **REF** K2-13-50-CE.

RIBO-zol-C nucleic acid extraction kit variant 100, **REF** K2-13-100-CE.

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

<i>Reagent</i>	<i>Description</i>	<i>variant 50</i>		<i>variant 100</i>	
		<i>Volume (ml)</i>	<i>Amount</i>	<i>Volume (ml)</i>	<i>Amount</i>
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	5.0	2 tubes

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

RIBO-zol-C 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).
- 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.
- Refrigerator for 2–8 °C with deep-freezer for ≤ -16 °C.
- Waste bin for used tips.
- Permanent pen for labeling.
- Thermostatic bath or dry block for tubes with controlled temperature and capable of incubating at 25-100 °C.

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:*
R20/21/22-32-52/53, S13-36/37-46-61



Xn

Solution B

Contains isoamyl alcohol: harmful (Xn). Risk and safety phrases:*
R10-20-37-66, S46



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

*R10: Flammable;

R20: Harmful if inhalation;

R22: Harmful if swallowed;

R32: Contact with acids liberates very toxic gas;

R34: Causes burns;

R37: Irritating to the respiratory system;

R38: Irritating to the skin;

R40: Limited evidence of a carcinogenic effect;

R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment;

R66: Repeated exposure can cause skin dryness or cracking;

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;

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

S13: Keep away from food, drink and animal feeding stuffs;

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;

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;

S61 Avoid release to the environment. Refer to special instructions/ Safety data sheets.

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-C nucleic acid extraction kit is recommended for **RNA** extraction and purification from biological materials.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. RNA Extraction

1. Prepare required number of 1.5 ml disposable tubes including one tube for Negative Control of Extraction (**C-**) and one tube for Positive Control of Extraction (**PCE**) (if it is provided with the amplification kit).
2. Add to each tube **10 µl** of **Internal Control (IC)** and then add **300 µl** of **Solution D**. Label the test tubes.
3. Add **100 µl** of prepared sample to the tubes with Internal control (IC) and Solution D using pipette tips with aerosol barriers.
4. Prepare Controls as follows:
 - a. Add **100 µl** of **Negative Control** (provided with the amplification kit) to the tube labeled **C-**.
 - b. Add **80 µl** of **Negative Control** (provided with the amplification kit) and **10 µl** of **Positive Control** (provided with the amplification kit) to the tube labeled **PCE**.
5. Tightly lock all tubes, stir on vortex, and incubate at 56 °C for 5 min occasionally stirring on vortex. Then centrifuge tubes at 10,000 rpm for 5 s (for removing drops from the internal surface of the tubes).
6. Add **30 µl** of **Solution E** into each tube, mix on vortex, then centrifuge the tubes at 10,000 rpm for 5 s.
7. Add **300 µl** of **Solution A** into each tube, mix on vortex, then centrifuge the tubes at 10,000 rpm for 5 s.
8. Add **100 µl** of **Solution B** into each tube, mix on vortex for 1-2 min (solution should become white), and place the tubes on ice (0–4 °C) for 10 min. Then centrifuge the tubes at 13,000 rpm for 10 min.
9. Solution should separate into 2 phases: bottom phase (phenolic), which contains proteins and DNA, and top phase (hydrous), which contains RNA. Interphase can appear as well. Carefully remove top phase (about 400 µl) and interphase (ensure that bottom phase is not disturbed) and transfer them in a clean 1.5 ml tube.

RNA extraction should be continued by using of second stage nucleic acid extraction kit. Follow the instruction manual. Start extraction from lysis step taking into account that Internal Control sample has already been added.

8.2. Amplification.

Different manufacturers offer PCR amplification kits. We recommend to use AmpliSens® PCR

amplification kits.



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.
- Degradation of the extracted nucleic acid. It's necessary to use plastic free from DNAses and RNAses.

False positives with extraction 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 Amplification areas.

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

10. TRANSPORTATION

RIBO-zol-C 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-C** nucleic acid extraction kit are to be stored at 2-8 °C, when not in use. They also must 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.

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 Quality Management System, each lot of **RIBO-zol-C**

nucleic acid extraction kit is tested against predetermined specifications to ensure consistent product quality.

14. KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Sufficient for
	<i>In vitro</i> diagnostic medical device		Expiration Date
	Version		Consult instructions for use
	Temperature limitation	PCE	Positive Control of Extraction
	Manufacturer	C-	Negative control of extraction
	Date of manufacture	IC	Internal control
	Authorised representative in the European Community		Toxic
			Harmful

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	
05.07.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
	Transportation	Phrase "for no longer than 5 days" was added