



For *in Vitro* Diagnostic Use

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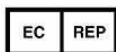
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# RIBO-zol-A

## nucleic acid extraction kit

### Instruction Manual

## AmpliSens®



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## 1. INTENDED USE

**RIBO-zol-A** 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-A** nucleic acid extraction kit is the reagents kit for rapid and efficient manual extraction and purification of RNA from various clinical materials. RNA extraction is based on separation of phenolic and hydrous phases. Hydrous phase, obtained after addition of Ribozol and Solution B, contains RNA. Sediment, containing purified RNA, is formed after addition of Solution C and Washing solution 3. RNA extracted from clinical samples may be used for PCR diagnostic tests.

## 3. CONTENT

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

**RIBO-zol-A** nucleic acid extraction kit variant 50, **REF** K2-2-50-CE.

**RIBO-zol-A** nucleic acid extraction kit variant 100, **REF** K2-2-100-CE.

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

Reagent	Description	Variant 50		Variant 100	
		Volume (ml)	Amount	Volume (ml)	Amount
Ribozol	colorless, clear liquid	37.5	1 vial	75	1 vial
Solution B	colorless, clear liquid	10	1 vial	20	1 vial
Solution C	colorless, clear liquid	22.5	1 vial	45	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-A** nucleic acid extraction kit variant 50 is intended for RNA extraction from 50 samples, including controls.

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



Use control samples – negative control of extraction (C-), internal control (IC), positive control of extraction (PCE) – for respective infectious agents, if their RNAs are to be analyzed.

## 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 16000 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 with temperature no less than minus 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 °C and 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, then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.



Xn

### Solution B

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



T

### Ribozol

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

**Ribozol**

Contains 2-mercaptoethanol: (T) Toxic, (N) Dangerous for the environment.

Risk and safety phrases:\*

R23/24/25-38-41-43-50/53, S26-36/37/39-45-60-61

**Solution C**

Contains isopropanol: Highly flammable (F), Irritant (Xi)

Risk and safety phrases: \*

R11-36-67, S7-16-24/25-26

**Washing Solution 3**

Contains ethanol: flammable. Risk phrase: \* R10

\*R10: Flammable;

R11: Highly flammable;

R20: Harmful if inhalation;

R22: Harmful if swallowed;

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;

R41 Risk of serious damage to eyes.

R43 May cause sensitization by skin contact.

R66: Repeated exposure can cause skin dryness or cracking;

R67: Vapours may cause drowsiness and dizziness;

R68: Possible risk of irreversible effect;

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

R50/53 Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

S 7: Keep container tightly closed;

S16: Keep away from sources of ignition - No smoking;

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;

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;

S60 This material and its container must be disposed of as hazardous waste.

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 is described in manufacturer's handbook [2]. It is recommended that this handbook is read before starting work.

**RIBO-zol-A** nucleic acid extraction kit is recommended for **RNA** extraction from clinical materials.

**7. PROTOCOL****7.1. RNA Isolation**

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)** (if it is provided for analysis of this infectious agent) and then add **750 µl** of **Ribozol**. Label the test tubes.
3. Add **100 µl** of prepared sample to the tubes with Internal control (IC) and Ribozol 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 **90 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 60 °C for 5 min in a thermostat. Mix on vortex. Then centrifuge tubes at 5000 rpm for 5 sec.
6. Add **110 µl** of **Solution B** into each tube, mix on vortex for 1 min and incubate in a freezer at the temperature between 2 and 8 °C.
7. Centrifuge the tubes at 13000 rpm for 10 min in a minicentrifuge. Carefully take out the tubes from minicentrifuge (do not shake). Solution should separate into 2 phases: bottom and top phase.
8. Thoroughly mix **Solution C**. 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**). Add **450 µl** of **Solution C** into each tube. Label the tubes.
9. Using tips with aerosol barrier carefully remove **top phase (about 450 µl)** (do not disturb interphase) and transfer it in a tube with **450 µl** of **Solution C**. Mix on vortex all clinical and control samples, then incubate them in a deep-freezer at the temperature not more than minus 16 °C for 20 min.
10. Centrifuge tubes at 13000 rpm for 10 min. Using vacuum aspirator and separate tip for each sample carefully remove the supernatant (do not disturb the sediment).
11. Add **1000 µl** of **Washing solution 3** in each tube. Tightly close the tubes and mix the content by the tube inverting. Centrifuge at 13000 rpm for 10 min. Using vacuum aspirator and separate tip for each sample completely remove the supernatant (do not disturb the sediment).
12. Transfer the tubes in a thermostat at 60 °C for 5 min for sediment predrying. Tube lids are to be opened. Sediment, containing purified RNA, can be stored at the temperature not more

than minus 16 °C for 1 week.

13. Add **20-50 µl** of **RNA-eluent** to the tubes. Mix on vortex for 2-3 min. RNA-samples are ready for reverse transcription and PCR.

Purified RNA can be stored at the temperature between 2 and 8 °C for 4 hours and at the temperature not more than minus 68 °C for 1 year.

### 7.2. 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.

### 8. 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'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 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.

### 9. STABILITY AND STORAGE

All components of the **RIBO-zol-A** nucleic acid extraction kit are to be stored between 2 and 8 °C (except for RNA-eluent), when not in use. All components of the **RIBO-zol-A** nucleic acid extraction kit are to be stable until the expiry date stated on the label.



RNA-eluent is to be stored at the temperature not more than minus 16 °C.

### 10. 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 State Institution of Science "Central Research Institute of Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

### 11. QUALITY CONTROL

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

### 12. EXPLANATION OF SYMBOLS

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Contains sufficient for <N> tests
	Authorised representative in the European Community.		Consult instructions for use
	Caution, consult accompanying documents		Internal Control
	Negative Control of Extraction		Positive Control of Extraction
	Harmful		Toxic
	Flammable		