CE

IVD

For Professional Use Only

RIBO-sorb

nucleic acid extraction kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of
Science “Central Research
Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

TABLE OF CONTENTS

1. INTENDED USE

RIBO-sorb nucleic acid extraction kit is intended for extraction and purification of RNA and DNA from clinical materials.

2. PRINCIPLE AND PROCEDURE

RIBO-sorb nucleic acid extraction kit is reagents kit for rapid and efficient manual extraction and purification of RNA from various biological materials. Lysis solution contains chaotropic agent (guanidine thiocyanate) that lyses cells and denaturates cell proteins. The nucleic acids are then sorbed on silica particles. RNA or DNA extracted from biological samples may be used for PCR diagnostic tests.

3. CONTENT

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

RIBO-sorb nucleic acid extraction kit variant 50, **REF** K2-1-Et-50-CE

RIBO-sorb nucleic acid extraction kit variant 100, **REF** K2-1-Et-100-CE

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

Reagent	***Description***	***variant 50***		***variant 100***	
		Volume (ml)	***Quantity***	***Volume (ml)***	***Quantity***
Lysis Solution	colorless clear liquid	22.5	1 vial	45	1 vial
Washing Solution 1	colorless clear liquid	20	1 vial	40	1 vial
Washing Solution 3	colorless clear liquid	50	1 vial	100	1 vial
Washing Solution 4	colorless clear liquid	20	1 vial	40	1 vial
Sorbent	white suspension	1.25	1 tube	1.25	2 tubes
RNA-buffer	colorless clear liquid	0.5	5 tubes	0.5	10 tubes

RIBO-sorb nucleic acid extraction kit variant 50 is intended for 50 reactions, including controls.

RIBO-sorb nucleic acid extraction kit variant 100 is intended for 100 reactions, 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)

- Tube racks
- 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
- 1.5 ml polypropylene sterile tubes
- Refrigerator for 2–8 °C
- 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 amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration 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 accordance 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 mucosa. If skin, eyes and mucosa 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	**Lysis Solution**, **Washing Solution 1**	Contains guanidine thiocyonate. Guanidine thiocyonate 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
	**Wash Solution 3, Wash Solution 4**	Contains ethanol: flammable. Risk phrase:* R10

*R10: Flammable;
R20/21/22: Harmful by inhalation, in contact with skin and if swallowed;
R32: Contact with acids liberates very toxic gas;
R36/37/38: Irritating to eyes, respiratory system and skin;
R42/43: May cause sensitization by inhalation and skin contact;
S13: Keep away from food, drink and animal feeding stuffs;
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;
S36: Wear suitable protective clothing;
S36/37: Wear suitable protective clothing and gloves;
S46: If swallowed, seek medical advice immediately and show the container or label.

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

- *plasma*
- *serum*
- *fecal extract*
- *cervical or urethral scrapes (swabs)*
- *urine*
- *secret of the prostate gland*
- *saliva*
- *throat or nasopharynx or fauces swabs (lavages)*
- *biopsy and autopsy materials after getting of the water phase*
- *ticks, mosquitoes and ectoparasites (lice and fleas) after getting of the water phase*

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1.RNA and DNA Extraction

1. **Lysis Solution** and **Washing Solution 1** (if stored at 2-8 ºC) should be heated at 60–65 ºC until the ice crystals disappear.
2. Prepare required number of 1.5 ml disposable polypropylene micro centrifuge tubes including one tube for Negative Control of Extraction (**Negative Control**, **C-**) and one tube for Positive Control of Extraction (**Positive Control** (RNA or DNA) , **PCE**, if provided with the amplification kit).
3. Add **5 µl** of **Internal Control** (if it is provided for analysis of this infectious agent) to each tube and then add **450 µl** of **Lysis Solution**. Label the test tubes.
4. Add **100 µl** of sample to the appropriate tube using pipette tips with aerosol barriers.
5. Prepare Controls as follows:
   5.1. Add **100 µl** of **Negative Control** (provided with the amplification kit) to the tube labeled **C-**.
   5.2. Add **90 µl** of **Negative Control** (provided with the amplification kit) and **10 µl** of **Positive Control** to the tube labeled **PCE**.
6. Tightly close all tubes and mix carefully on vortex for 7-10 s.
7. Centrifuge all tubes for 5 s at 5000g (for removing drops from internal surface of the lids).
8. Thoroughly resuspend **Sorbent** on vortex and add **25 µl** of it into each test tube.
9. Vortex tubes for 5-7 s, place in a rack for 60 s, once again mix on vortex for 5-7 s and incubate all tubes for 5 min at room temperature.
10. Centrifuge all tubes for 30 s at 10,000 g (for sorbent precipitation) and carefully discard supernatant from every tube without disturbing the pellet using vacuum aspirator. Use a new tip for every tube.
11. Add **400 µl** of **Washing Solution 1** into each tube. Vortex vigorously (until sorbent is fully resuspended) and centrifuge for 30 s at 10,000 g. Using vacuum aspirator, carefully remove and discard supernatant from each tube without disturbing the pellet. Use a new tip for every tube.
12. Add **500 µl** of **Washing Solution 3** to each tube. Mix by Vortex vigorously and centrifuge for 30 sec at 10,000g. Carefully remove and discard supernatant from each tube without disturbing the pellet using vacuum aspirator. Use a new tip for every tube.
13. Repeat step 12.
14. Add **400 µl** of **Washing Solution 4** to each tube. Mix by Vortex vigorously and centrifuge for 30 sec at 10,000g. Carefully remove and discard supernatant from each tube without disturbing the pellet using vacuum aspirator. Change tips between tubes.
15. Incubate all tubes with open caps for 12-15 min at 60 °C (for sorbent predrying).

16. Resuspend the pellet in **50 µl** of **RNA-buffer**, using tip with aerosol barrier (RNAses-free)**.** Mix on vortex vigorously. Incubate for 2-3 min at 60 °C.
17. Once again mix on vortex and centrifuge the tubes for 1 min at maximum speed (12,000-16,000 g).

The supernatant contains purified RNA and DNA and is ready to use in reverse transcription reaction or PCR amplification. Be careful not to collect sorbent while taking the solution of DNA and RNA off. If solution is muddy, centrifuge the tube to precipitate the sorbent.

It is recommended to conduct the reverse transcription reaction immediately after extraction and purification of RNA. The amplification can be performed in the day of extraction.

The purified RNA can be stored:

- at 2-8 °C for 4 hours;
- at not more than minus 68 °C for 1 year (carefully transfer supernatant into new sterile tube without disturbing the pellet).

If using the RNA samples for a diagnostic assay, follow the instructions given by the manufacturer.

8.2. Amplification

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

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's necessary to use a new sample, store samples appropriately.
- Loss of nucleic acid deposit. Carefully draw off the washing solution and try not to remove the sorbent.
- 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-sorb nucleic acid extraction kit should be transported at 2–8 ºC for no longer than 5 days.

11. STABILITY AND STORAGE

All components of **RIBO-sorb** nucleic acid extraction kit are to be stored at 2-8ºC, when not in use. All components of RIBO-sorb nucleic acid extraction kit are to be stable until labeled expiration date. 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 Total Quality Management System, each lot of RIBO-sorb nucleic acid extraction kit is tested against predetermined specifications to ensure consistent product quality.

14. KEY TO SYMBOLS USED

REF	Catalogue number
LOT	Batch code
IVD	*In vitro* diagnostic medical device
VER	Version
	Temperature limitation
	Manufacturer
	Date of manufacture
EC REP	Authorised representative in the European Community

	Caution
	Sufficient for
	Expiration Date
	Consult instructions for use
	Harmful
	Flammable

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”