



IVD

For *in Vitro* Diagnostic Use

RIBO-sorb

nucleic acid extraction kit

Instruction Manual

AmpliSens®

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1. INTENDED USE.

RIBO-sorb nucleic acid extraction kit is intended for use to extract and purify 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. CONTENTS.

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)	Amount	Volume (ml)	Amount
Lysis Solution	colorless, clear fluid	22.5	1 vial	45	1 vial
Washing Solution 1	colorless, clear fluid	20	1 vial	40	1 vial
Washing Solution 3	colorless, clear fluid	50	1 vial	100	1 vial
Washing Solution 4	colorless, clear fluid	20	1 vial	40	1 vial
Sorbent	white suspension	1.25	1 tube	1,25	2 tubes
RNA-buffer	colorless, clear fluid	0.5	5 tubes	0.5	10 tubes

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

RIBO-sorb nucleic acid extraction kit variant 100 is sufficient for 100 reactions, including controls.

4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES.

- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile RNase-free pipette tips with aerosol filters (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
- Refrigerator for 2–8 °C
- Deep-freezer with temperature no less than minus 16 °C.
- Reservoir for disposed tips.
- Permanent pen for labeling
- Thermostat for tube 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 filters and put the new tip for every procedure.
- Store and handle amplicons separately from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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 specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucose membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- 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.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area in which the previous step was performed.



Xn

Lysis Solution, Washing Solution 1

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

**Washing Solution 3, Washing 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 feedingstuffs;
 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 is 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. PROTOCOL.**7.1. RNA and DNA Isolation**

1. **Lysis Solution** and **Washing Solution 1** (if stored at temperature between 2 and 8 °C) should be heated at 60– 65 °C 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 sec.
7. Centrifuge all tubes for 5 sec 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 sec, place in a rack for 60 sec, once again mix on vortex for 5-7 sec and incubate all tubes for 5 min at room temperature.
10. Centrifuge all tubes for 30 sec 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 sec 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 between 2 and 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.

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 instruction.

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 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.

9. STABILITY AND STORAGE.

All components of RIBO-sorb nucleic acid extraction kit are to be stored at between 2°C and 8°C, when not in use. All components of RIBO-sorb nucleic acid extraction kit are to be stable until labeled expiration date.

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

12. EXPLANATION OF SYMBOLS.



Manufacturer



Use by



For *in Vitro* Diagnostic Use



Catalogue number



Contains sufficient for <n> tests



Consult instructions for use



For working with Rotor-Gene™ 3000/6000



Positive control



Temperature limitation



Batch code



Version



Internal Control complex



Authorized representative in the European Community.



Caution, consult accompanying documents



For working with iQ5, iQ iCycler



Negative control