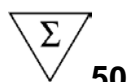


Ribo-Sorb

Nucleic acid extraction kit for the isolation and purification of
RNA/DNA from clinical materials

USER MANUAL

REF K-2-1



50

REF K-2-1/100



100

NAME

Ribo-Sorb

INTENDED USE

The **Ribo-Sorb** nucleic acid extraction kit is intended for the isolation and purification of RNA/DNA from plasma, serum, whole blood, liquor, amniotic liquid, tissue, urine, feces, bronco aspirates and other biological materials.

MATERIALS PROVIDED

Ribo-Sorb (K-2-1)

- **Lysis Solution**, 22,5 ml;
- **Washing Solution**, 20 ml;
- **Sorbent**, 1,25 ml.
- **RNA-eluent**, 5 x 0,5 ml.

Contains reagents for 50 tests.

Ribo-Sorb (K-2-1/100)


- **Lysis Solution**, 45 ml;
- **Washing Solution**, 40 ml;
- **Sorbent**, 2 x 1,25 ml.
- **RNA-eluent**, 10 x 0,5 ml.

Contains reagents for 100 tests.

MATERIALS REQUIRED BUT NOT PROVIDED

- Biological cabinet
- Desktop microcentrifuge for "eppendorf" type tubes
- 60°C ± 2°C dry heat block
- Vortex mixer
- Pipettors (capacity 5-40 µl; 40-200 µl; 200-1000 µl) with aerosol barrier
- 1,5 ml polypropylene sterile tubes (Sarstedt, QSP, Eppendorf)
- Disposable gloves, powderless
- Tube racks
- Freezer, Refrigerator
- 70% Ethanol (freshly prepared mixture of reagent grade 96% ethanol and distilled water)
- Acetone

WARNINGS AND PRECAUTIONS

-  Lysis Solution contains guanidine thiocyanate. Guanidine thiocyanate is harmful if inhaled, or comes in contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/37/38; S: 36/37/39).
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
- Do not pipette by mouth.
- 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 Biosafety Level 2 or other 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 mucous 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 where you performed previous step.

STORAGE AND SHIPPING

Ribo-Sorb can be stored at 2-25°C storage temperature. Reagent will crystallize upon storage at 2-8°C.

Ribo-Sorb can be shipped at room temperature.

STABILITY

Ribo-Sorb is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. All components of the Ribo-Sorb nucleic acid extraction kit are stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

SPECIMEN COLLECTION, STORAGE AND TRANSPORT

Ribo-Sorb Kit can isolate RNA/DNA from:

- *whole blood* collected in either ACD or EDTA tubes;
- *serum* collected blood in Serum Separator tubes;
- *plasma* collected blood in ACD or EDTA tubes;
- *bone marrow aspirate* collected in EDTA tube;
- *liquor* stored in "Eppendorf" tube;
- *lacrima liquid* stored in "Eppendorf" tube;
- *amniotic liquid* stored in "Eppendorf" tube;
- *sinovial liquid* stored in "Eppendorf" tube;
- *peritoneal and pleuric versament* stored in "Eppendorf" tube;
- *tissue* homogenized with mechanical homogenizer and dissolved in PBS sterile;
- *urine (sediment)*;
- *prostatic liquid* stored in "Eppendorf" tube;
- *seminal liquid*: transfer about 30 µl of seminal liquid to a polypropylene tube (1,5 ml) and add 70 µl of sterile saline solution;
- *sputum*: add 1 volume of sputum to 1 volumes of β-mercaptoethanol 0,1M (reagent not provided. β-mercaptoethanol is a toxic reagent, please follow the supplier MSDS). Vortex well and incubate at room temperature for 30 min, vortexing periodically. Transfer 1,0 ml of clinical material to a sterile polypropylene tube (1,5 ml) and centrifuge at 10000g/min for 10 min. Discard the supernatant and leave about 100 µl of solution for DNA extraction;
- *bronco aspirate*: transfer 1,0 ml to a polypropylene tube (1,5 ml) and centrifuge at 10000g/min for 10 min. Discard the supernatant and leave about 100 µl of solution for DNA extraction;
- *feces*: prepare required quantity of 1,5 ml polypropylene tubes with 1,0 ml of Saline Solution. Add to each tube 0,1 g of feces. Vortex vigorously to get a homogeneous suspension. Centrifuge for 5 min at 7-12000g and use the supernatant for the extraction of the RNA

It is recommended to process samples immediately after collection. Store samples at 2–8 °C for no longer than 24 hours, or freeze at –20/80°C.

SPECIMEN AND REAGENT PREPARATION

1. **Lysis Solution** and **Washing Solution** (in case of their storage at +2-8°C) should be warmed up to 60–65°C until disappearance of ice crystals.
2. Prepare required quantity of 1.5 ml polypropylene tubes including one tube for **Negative Control of Extraction** and one tube for **RNA Positive Control of Extraction** (if provided with the amplification kit).
3. Add to each tube **5 µl** of **Internal Control** and **450 µl Lysis Solution**.
4. Add **100 µl** of **Samples** to the appropriate tube.
5. Prepare Controls as follows:
 - add **100 µl** of **C– Negative Control** (provided with the amplification kit) to each of the two control tubes.
 - add **10 µl** of **RNA Control C+** to the tube labeled *Cpos*.
6. Vortex the tubes and centrifuge for 7-10 sec.
7. Vortex vigorously **Sorbent** and add **25 µl** to each tube.
8. Vortex for 5-7 sec and incubate all tubes for 10 min at room temperature. Vortex periodically.
9. Centrifuge all tubes for 30 sec at 10000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
10. Add **400 µl** of **Washing Solution** to each tube. Vortex vigorously and centrifuge for 30 sec at 10000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
11. Add **500 µl** of **Ethanol 70%** to each tube. Vortex vigorously and centrifuge for 30 sec at 10000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
12. Repeat step 11.
13. Add **400 µl** of **Acetone** to each tube. Vortex vigorously and centrifuge for 30 sec at 10000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
14. Incubate all tubes with open cap for 10 min at 60°C.
15. Resuspend the pellet in **50 µl** of **RNA-eluent**. Incubate for 10 min at 60°C and vortex periodically.
16. Centrifuge the tubes for 1 min at maximum speed (12000-16000 g). The supernatant contains RNA/DNA ready for use. The amplification can be performed on the same day of extraction. If this is not possible, the RNA preparations can be stored at -80°C for up to one month.

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. Use plastic free from DNAses and RNAses

False positives with extraction product:

- Contamination during sample extraction. Open one test tube at time. Avoid spilling the contents of the test tube, always change tips. Use only filter tips during the extraction procedure. Change tips between tubes.
- Contamination of the reagents prepared for the step. Repeat the test with the new set of reagents.
- Contamination of the extraction zone by amplicons. Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol, wash lab coats, replace test tubes and tips in use. Use different laboratory coats in different Amplification areas.

KEY TO SYMBOLS USED



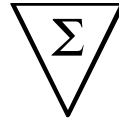
List Number



Caution!



Lot Number



Contains sufficient
for <n> tests



For *in Vitro* Diagnostic
Use



Version



Store at



Expiration Date



Manufacturer



Warning



Consult instructions for
use



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