CE

IVD

For Professional Use Only

RIBO-prep

nucleic acid extraction kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

RIBO-prep nucleic acid extraction kit is intended for extraction and purification of total RNA/DNA from clinical materials (peripheral blood plasma, cerebrospinal and amniotic fluid, nasal and oropharyngeal swabs, and saliva).

2. PRINCIPLE OF NUCLEIC ACID EXTRACTION

RIBO-prep nucleic acid extraction kit is reagents kit for rapid and efficient manual extraction and purification of RNA from various biological materials. Solution for lysis contains a chaotropic agent (guanidine thiocyanate), which lyses cells and denatures cell proteins. Nucleic acids are then precipitated in isopropanol. RNA or DNA extracted from biological samples may be used for PCR diagnostic tests.

3. CONTENT

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

RIBO-prep nucleic acid extraction kit variant 50, **REF** K2-9-Et-50-CE

RIBO-prep nucleic acid extraction kit variant 100, **REF** K2-9-Et-100-CE

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

Reagent	***Description***	***variant 50***		***variant 100***	
		Volume, ml	***Quantity***	***Volume, ml***	***Quantity***
Solution for Lysis	blue clear liquid[1]	15	1 vial	30	1 vial
Solution for Precipitation	colorless clear liquid	20	1 vial	40	1 vial
Washing Solution 3	colorless clear liquid	25	1 vial	50	1 vial
Washing Solution 4	colorless clear liquid	10	1 vial	20	1 vial
RNA-buffer	colorless clear liquid	1.2	4 tubes	1.2	8 tubes

RIBO-prep nucleic acid extraction kit variant 50 is intended for 50 RNA/DNA extraction, including controls.

RIBO-prep nucleic acid extraction kit variant 100 is intended for 100 RNA/DNA extraction, including controls.

4. ADDITIONAL REQUIREMENTS

- Disposable powder-free gloves and laboratory coat.

[1] If Solution for Lysis is stored at 2-8 °C, a crystalline precipitate may form.

- 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 rpm).
- 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 another 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 and 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.

Solution for Lysis has an unpleasant smell. Work with this solution should be performed in a biological cabinet.

 Xn	**Solution for Lysis**	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**	Contains ethanol: flammable. Risk phrase:* R10
 Xi	**Solution for Precipitation**	Contains 2-propanol: Highly flammable. Irritant. Risk and safety phrases:* R11-36-67, S7-16-24/25-26:

***Risk phrases:**

R10	Flammable;
R11	Highly 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;
R36	Irritating to the eyes;
R42/43	May cause sensitization by inhalation and skin contact;
R67	Vapors may cause drowsiness and dizziness S13: Keep away from food, drink and animal feeding-stuffs;

Safety phrases:

S7	Keep container tightly closed
S16	Keep away from sources of ignition - No smoking
S22	Do not breathe dust;
S23	Do not breathe spray;
S24	Avoid contact with skin;
S24/25	Avoid contact with skin and eyes
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-prep nucleic acid extraction kit is recommended for **RNA and DNA** extraction and purification from:

- *blood plasma;*
- *cerebrospinal fluid;*
- *amniotic fluid;*
- *saliva;*
- *nasal or feces swabs;*

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. RNA/DNA extraction

1. **Solution for Lysis,** if stored at 2–8 °C, should be heated at 60–65 °C until the crystals disappear.
2. Take the required number of 1.5-ml disposable tubes with tightly closable caps including one tube for Negative Control of Extraction (**C–**) and one tube for Positive Control of Extraction (**PCE**). Add **10 µl** of **Internal Control** (if it is provided for analysis of this infectious agent) to each tube and then add **300 µl** of **Solution for Lysis**. Label test tubes.
3. Add **100 µl** of prepared samples to the tubes with **Solution for Lysis** and **Internal Control** (if used) using pipette tips with aerosol barriers. Add **100 µl** of **Negative Control** to the tube labeled **C–**. Add **90 µl** of **Negative Control** and **10 µl** of **Positive Control** (if it is provided for analysis of the pathogen) to the tube labeled **PCE**.
4. Mix the contents of the tubes thoroughly by vortexing, then centrifuge tubes for 5 s to be sure there are no drops on the cap, and incubate them at 65 °C for 5 min.
5. Add **400 µl** of **Solution for Precipitation** and mix by vortexing.
6. Centrifuge all tubes for 5 min at 13,000 rpm.
7. Carefully remove the supernatant without disturbing the pellet using a vacuum aspirator and 200-µl tips. Use a new tip for each tube.
8. Add **500 µl of Washing Solution 3** to each tube, tightly close the tubes and turn them carefully upside down and back 3–5 times to wash the pellet. This procedure can be

performed simultaneously for all the tubes: cover the tubes placed in a rack with a lid or another rack, press them, and turn the rack.

9. Centrifuge all tubes at 13,000 rpm for 1–2 min.
10. Carefully remove the supernatant without disturbing the pellet using a vacuum aspirator and 10-µl tips. Use a new tip for each tube.
11. Add **200 µl of Washing Solution 4** to each tube, tightly close the tubes and turn them carefully upside down and back 3–5 times to wash the pellet.
12. Centrifuge all tubes at 13,000 rpm for 1–2 min.
13. Carefully remove the supernatant without disturbing the pellet using a vacuum aspirator and 10-µl tips. Use a new tip for each tube.
14. Incubate all tubes with open caps at **65 °C for 5 min** (to dry the pellet).
15. Add **50 µl** of **RNA buffer** into each tube. Mix the tubes by vortex. Then incubate them at 65 °C for 5 min occasionally stirring by vortex. Elution volume can be increased up to 90 µl.
16. Centrifuge the tubes at 13,000 rpm for 1 min.

The supernatant contains purified RNA and DNA. Samples are ready for reverse transcription reaction or PCR amplification.

The purified RNA/DNA can be stored

- at 2–8 °C for 24 h;
- at not more than minus 16 °C for 1 year.

8.2. Amplification

It is 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.

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 is necessary to use a new sample. Store samples under appropriate conditions.
- Loss of nucleic acid pellet. Carefully discard the washing solution trying not to disturb the nucleic acid pellet.
- Degradation of the extracted nucleic acid. It is necessary to use DNase- and RNase-free plastic.

False positives with extraction product:

- Contamination during sample extraction. It is necessary to open one test tube at a time. Avoid spilling the contents of the test tube. Always change tips.
- Contamination of the reagents prepared for the step. It is necessary to repeat the test.
- Contamination of the extraction zone by amplicons. It is necessary to clean surfaces and instruments using aqueous detergents, wash lab coats, and replace test tubes and tips in use. Use different laboratory coats in different zones.

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

10. TRANSPORTATION

RIBO-prep nucleic acid extraction kit should be transported at 2–25 ºC for no longer than 5 days.

11. STABILITY AND STORAGE

All components of RIBO-prep nucleic acid extraction kit are to be stored at 2–8 °C when not in use. All components of the RIBO-prep nucleic acid extraction kit are stable until the expiration date 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. Handbook “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-prep** nucleic acid extraction kit is tested against predetermined specifications to ensure consistent product quality.

14. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code	Σ	Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Harmful
	Manufacturer		Flammable
	Date of manufacture	EC REP	Authorised representative in the European Community

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
10.12.10	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
	Content	The color of Solution for Lysis was changed into blue
		The volume of Washing Solution 3 was changed into 25 ml (for variant 50)
		The reference «If Solution for Lysis is stored at 2-8 °C, a crystalline precipitate may form» was added
04.07.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
06.09.11 RT	8. PROTOCOL 8.1. RNA/ DNA extraction	Procedure of extraction was corrected (heating at 65 °C for 5 min was added in Section 8.1, article 4).
12.07.12 BM	5. General precautions	Information about an unpleasant smell of Solution for Lysis and the necessity to work in a biological cabinet was added