CE

IVD

For Professional Use Only

DNA-sorb-C

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

DNA-sorb-C nucleic acid extraction kit is intended for the extraction and purification of DNA from clinical material, food and animal feeding stuff.

2. PRINCIPLE OF NUCLEIC ACID EXTRACTION

DNA-sorb-C nucleic acid extraction kit is reagents kit for rapid and efficient manual extraction and purification of DNA from various biological materials. **Lysis Reagent Buffer** and **Washing Solution 1** contain chaotropic agents (guanidine chloride and guanidine thiocyanate), which lyse cells and denature cell proteins, respectively. The nucleic acids are then sorbed on silica particles. DNA extracted from biological samples may be used for PCR diagnostic tests.

3. CONTENTS OF THE KIT

DNA-sorb-C nucleic acid extraction kit is produced in 1 form:

DNA-sorb-C nucleic acid extraction kit variant 50, **REF** K1-6-50-CE

DNA-sorb-C nucleic acid extraction kit variant 50 includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
Lysis Reagent Buffer	colorless clear fluid	20	1 vial
Lysis Reagent	colorless clear fluid	0.85	1 tube
Washing Solution 1	colorless clear fluid	15	1 vial
Washing Solution 2	colorless clear fluid	50	1 vial
Universal Sorbent	white suspension	1.25	1 tube
TE-buffer for DNA elution	colorless clear fluid	5.0	1 tube

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

4. ADDITIONAL REQUIREMENTS

- Disposable powder-free gloves and laboratory coat.
- 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 sterile polypropylene tubes.
- Refrigerator for 2–8 °C.
- Waste bin for used tips.
- Permanent pen for labeling.
- Thermostatic bath or dry block for tubes with controlled temperature 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 filters 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.
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- 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 compliance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills with a disinfectant, such as 0.5 % sodium hypochlorite, or another suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucosa membranes. If these solutions come into contact, immediately flush with water and 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.

 Xn	**Lysis Reagent Buffer, Washing Solution 1**	Contains guanidine chloride. Guanidine chloride 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 2**	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 are described in manufacturer's handbook [1]. It is recommended to read this handbook before starting work.

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

- microbiopsy material of skin, mucous membrane (urogenital system, gastrointestinal tract, bronchi), and parenchymal organs (liver or spleen aspirate) as well as homogenized tissue. **DNA-sorb-C** nucleic acid extraction kit is sufficient for extraction from 50 biopsy specimens (10–25 mm^3) or 50% tissue homogenates in volume no more than 50 µl.
- food, bioactive food additives, animal feeding stuff, plant stock.

6.1. *Microbiopsy material*

Skin, mucous, or parenchymal organ biopsy specimen which size is 10–25 mm^3 should be placed into a tube which contains 0.2 ml of saline solution or transport

medium.

Storage of samples:

– 2–8 ºC for 5–6 h;

– at ≤ –16 ºC for 1 month.

Prior to use, thaw the tube with the specimen and centrifuge it at 8,000 rpm for 5 min. Then transport medium should be carefully removed without disturbing the pellet.

6.2. *Macrobiopsy material*

Parenchymal organ biopsy specimen which size is more than 50 mm^3 should be placed into a container or a tube which contains 0.2 ml of saline or transport medium.

Storage of samples:

– at 2–8 ºC for 5-6 h;

– at ≤ –16 ºC for 1 month.

Prior to use, thaw the specimen and place it in a porcelain mortar. Add an equal volume of saline, PBS, or transport medium. Thoroughly homogenize the tissue with a pestle. Take 50µl of the suspension for DNA extraction. Transfer the rest of the sample to a clean tube and store at minus 40 ºC for 1 month.

Food, bioactive food additives, animal feeding stuff, and plant stock should be treated as described in “PLANT-SCREEN” PCR kit instruction manual (produced by FBIS CRIE).

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Extraction

Isolation from biopsy material

1. **Lysis Reagent Buffer** and **Washing Solution 1** (if stored at 2–8 ºC) should be heated to 60–64 ºC until disappearance of ice crystals.
2. In each tube which contains biopsy specimen or 50% homogenate add **400 µl** of **Lysis Reagent Buffer** and **17 µl** of **Lysis Reagent** using tips with aerosol filter. Mix well.
3. Incubate the tubes at 60 ºC for 1 h under periodic stirring on vortex (5 times every 10–12 min). Incubation at 60 ºC for 12 h is allowed as well.
4. Centrifuge the tubes at 12,000–14,000 rpm for 5 min.
5. Carefully transfer supernatant (about **200-350 µl**) using tips with aerosol filter and transfer into clean tubes.
6. Centrifuge the tubes at 5,000 rpm for 5 s.
7. Thoroughly resuspend **Universal Sorbent** on vortex. Into each test tube add **25 µl** of

Universal Sorbent. Carefully mix the tubes on vortex then leave them in a rack for 10-15 min stirring every 2 min.

8. Centrifuge the tubes at 5,000 rpm for 1 min. Remove the supernatant using vacuum aspirator and a separate tip for every sample.
9. Add **300 µl** of **Washing Solution 1,** stir on vortex until sorbent is fully resuspended. Centrifuge the tubes at 5,000 rpm for 1 min. Remove supernatant using vacuum aspirator without disturbing the pellet using a vacuum aspirator. Use a new tip (without aerosol barrier) for every tube.
10. Add **500 µl** of **Washing Solution 2,** stir on vortex until sorbent is completely resuspended. Centrifuge the tubes at 10,000 rpm for 1 min. without disturbing the pellet using a vacuum aspirator. Use a new tip (without aerosol barrier) for every tube.
11. Repeat washing as described in step 10. Remove supernatant completely.
12. Incubate the tubes with open caps at 64–65 ºC for 5–10 min (for sorbent drying).
13. Add **50-100 µl** of **TE-buffer for DNA elution** (depending on size of a sample (10–25 mm^3)). Stir on vortex. Incubate tubes at 64–65 ºC for 5–10 min; vortex occasionally while incubating (one time per min).
14. Centrifuge tubes at 12,000–14,000 rpm for 1 min. The supernatant contains purified DNA and is ready for PCR amplification. Be careful not to collect sorbent while taking the solution of DNA off. If solution is muddy, centrifuge the tube to precipitate the sorbent.

Isolation from food, bioactive food additives, animal feeding stuff and plant stock

1. **Lysis Reagent Buffer and Washing Solution 1** (if stored at 2-8ºC) should be heated to at 64 ºC until disappearance of ice crystals.
2. Prepare required quantity of 1.5 ml tubes including one tube for Negative Control of Extraction (**Negative Control, C-)**. Add samples into the tubes. Into the tube for Negative Control of Extraction add **100 µl** of **Negative Control.**
3. Add **400 µl** of **Lysis Reagent Buffer** and **17 µl** of **Lysis Reagent** into each tube. Stir thoroughly.
4. Incubate the tubes at 64 ºC for 1 hour occasionally stirring on vortex (5 times every 10-12 min).
5. Centrifuge the tubes at 12,000-14,000 rpm for 5 min.
6. Carefully transfer supernatant (about **200-350 µl**) using tips with aerosol filter into clean tubes. Ensure that suspended particles and oil drops are not transferred.
7. Centrifuge the tubes at 5,000 rpm for 5 sec.
8. Thoroughly resuspend **Universal Sorbent** on vortex. Into each test tube add **25 µl** of **Universal Sorbent.** Carefully mix the tubes on vortex then place them in a rack for 10-

15 min stirring every 2 min.

9. Centrifuge the tubes at 5,000 rpm for 1 min. Remove supernatant using vacuum aspirator without disturbing the pellet using a vacuum aspirator. Use a new tip (without aerosol barrier) for every tube.
10. Add **300 µl** of **Washing Solution 1,** stir on vortex until sorbent is fully resuspended. Centrifuge the tubes at 5,000 rpm for 1 min. Remove supernatant using vacuum aspirator without disturbing the pellet using a vacuum aspirator. Use a new tip (without aerosol barrier) for every tube.
11. Add **500 µl** of **Washing Solution 2,** stir on vortex until sorbent is fully resuspended. Centrifuge the tubes at 10,000-12,000 rpm for 1 min. Remove supernatant using vacuum aspirator without disturbing the pellet using a vacuum aspirator. Use a new tip (without aerosol barrier) for every tube.
12. Repeat washing as described in step 11. Remove supernatant completely.
13. Incubate the tubes with open caps at 64 ºC for 5-10 min (for sorbent drying).
14. Add **50 µl** of **TE-buffer for DNA elution.** Stir on vortex. Incubate tubes at 64 ºC for 5-8 min; vortex occasionally while incubating (1 time per minute).
15. Centrifuge tubes at 12,000-14,000 rpm for 1 min. The supernatant contains purified DNA and is ready for PCR amplification. Be careful not to collect sorbent while taking the DNA solution for analysis. If the solution is muddy, centrifuge the tube to precipitate the sorbent.

The purified DNA could be stored:

- at 2–8 °C for 1 week;
- at ≤ –16 °C for 1 year.

If using the DNA samples in a diagnostic assay, follow the instructions provided by the manufacturer.

8.2. Amplification

It is recommended to use AmpliSens® PCR amplification kits.

Please carry out amplification according to the manufacturer's 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. Use a new sample, store samples appropriately.

- Loss of nucleic acid deposit. Carefully draw off the wash solution and try not to remove the nucleic acid deposit.
- Degradation of the extracted nucleic acid. DNase-free and RNase-free plastic ware should be used.

False positives with extraction product:

- Contamination during sample extraction. One test tube at a time should be opened. 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, replace test tubes and tips in use. Use different laboratory coats in different areas.

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

10. TRANSPORTATION

DNA-sorb-C nucleic acid extraction kit should be transported at 2–8 ºC for no longer than 5 days.

11. STABILITY AND STORAGE

All components of DNA-sorb-C nucleic acid extraction kit (except for Lysis Reagent) are to be stored at 2–25 ºC when not in use. They 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.

Lysis Reagent should be stored at 2–8 °C.

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 Quality Management System, each lot of DNA-sorb-C

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		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Flammable
EC REP	Authorised representative in the European Community		Harmful
	Manufacturer		Date of manufacture
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”		

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
01.07.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”