DNA-sorb-C nucleic acid extraction kit

CE IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit	**FBIS CRIE**	Federal Budget Institute of Science "Central Research Institute for Epidemiology"
	Manufacturer		GHS07: Exclamation mark
	Date of manufacture		GHS02: Flame
EC REP	Authorized representative in the European Community		GHS05: Corrosion
			GHS08: Health hazard

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.

Indications and contra-indications for use of the reagent kit
DNA extraction is used in preanalytical stage of in vitro diagnostics by nucleic acid amplification techniques (NAT).

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. CONTENT

DNA-sorb-C nucleic acid extraction kit is produced in 1 form:
variant 50, REF K1-6-50-CE.
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	suspension from white to dark beige colour	1.25	1 tube
TE-buffer for DNA elution	colorless clear fluid	5	1 tube

Variant 50 is intended for 50 reactions, including controls.

4. ADDITIONAL REQUIRMENTS

- Vacuum aspirator with flask for removing supernatant.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl and 1000 µl).
- Vortex mixer.
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RCF max.16,000 x g).
- PCR box.
- Thermostat for tubes with controlled temperature for 25-100 °C.
- Tube racks.
- Disposable 1.5-ml sterile polypropylene tubes.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile DNase- RNase-free pipette filter tips and use new tip for every procedure.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- 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.
- 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 using a disinfectant, such as 0.5% sodium hypochlorite, or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA/RNA extraction.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area where the previous step was performed.
- 1.5 ml and 5-ml disposable polypropylene sterile tubes with screw or tight-fitting caps.
- Thermostat for tubes with controlled temperature and capable of incubating at 25-100 °C. Tube racks.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

Washing Solution 1 Danger	Contains substance: guanidine thiocyanate. H302: Harmful if swallowed. H312: Harmful in contact with skin. H314: Causes severe skin burns and eye damage. H332: Harmful if inhaled. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P260: Do not breathe vapours. P264: Wash your hands thoroughly after handling. P273: Avoid release to the environment. P302+P352: IF ON SKIN: Wash with plenty of water P501: Dispose of contents in accordance with national regulation.
Washing Solution 2 Warning	Contains substance: Isopropyl acoholl H226: Flammable liquid and vapour. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P261: Avoid breathing vapours. P264: Wash your hand thoroughly after handling. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. P403+P233: Store in a well ventilated place. Keep container tightly closed. P501: Dispose of contents in accordance with national regulation.
Universal Sorbent Warning	Contains substance: Celite® H373: May cause damage to organs through prolonged or repeated exposure P260: Do not breathe dust/fume/gas/mist/vapours/spray. P314: Get medical advice if you feel unwell. P501: Dispose of contents in accordance with national regulation.
Lysis Reagent Buffer Warning	Contains substance: guanidine chloride H315: Causes skin irritation. H319: Causes serious eye irritation. P264: Wash your hands thoroughly after handling. P280: Wear protective gloves/protective clothing/eye protection/face protection. P302 + P352: IF ON SKIN: Wash with plenty of water P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. P362: Take off contaminated clothing. P501: Dispose of contents in accordance with national regulations.

6. SAMPLING AND HANDLING

NOTE: Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in manufacture's handbook [1]. It is recommended to read this handbook before starting of the work.

DNA-sorb-C nucleic acid extraction kit is intended 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.

Sampling

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 the temperature not more than minus 16 °C 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.

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 the temperature not more than minus 16 °C 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.

NOTE: 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).

Interfering substances and limitations of using test material samples

The information about potential interfering substances and limitations of using test material samples is specified in the Instruction Manual of the PCR kit.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1 DNA Extraction

Extraction from biopsy material

1. Warm **Lysis Reagent Buffer** and **Washing Solution 1** (if stored at 2–8 ºC) at 60-64 ºC until the crystals disappear.
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 thoroughly**.**
3. Incubate the tubes at 60 ºC for 1 hour with periodic stirring on vortex (5 times every 10–12 min). Incubation at 60 ºC for 12 hours 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 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.** Vortex thoroughly the tubes and 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,** mix 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 (to dry the pellet).
13. Add **50-100 µl** of **TE-buffer for DNA elution** (depending on size of a sample (10–25 mm^3)). Mix 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. The samples are 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.

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

1. Warm **Lysis Reagent Buffer** and **Washing Solution 1** (if stored at 2–8 ºC) at 60-64 ºC until the crystals disappear.
2. Take the required quantity of 1.5 ml tubes including one tube for Negative Control of Extraction **(Negative Control (C-))**. Add samples into the tubes. Add **100 µl** of **Negative Control (C-)** into the tube for Negative Control of Extraction (C-)**.**
3. Add **400 µl** of **Lysis Reagent Buffer** and **17 µl** of **Lysis Reagent** into each tube. Mix the tubes.
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 filter) for each 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 the temperature not more than minus 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.

NOTE: 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 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's 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's 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 areas.

If you have any questions or if you 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 °C 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 also must be stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: Lysis Reagent is to be stored at 2–8 °C.

12. REFERENCES

1. R Boom, C J Sol, M M Salimans, C L Jansen, P M Wertheim-van Dillen and J van der Noordaa; "Rapid and simple method for purification of nucleic acids. "J. Clin. Microbiol. March 1990 vol. 28 no. 3 495-503.
2. Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being.

13. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **DNA-sorb-C** nucleic acid extraction kit has been tested against predetermined specifications to ensure consistent product quality.

Please contact our Authorized representative in the European Community if side effects, undesirable reactions, facts and circumstances that pose a threat to the life and health of citizens and medical workers are identified during the use of the reagent kit.

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
	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"
31.03.15 ME	5. General precautions, 14. Key to symbols used	Information about hazards was corrected
10.10.17 PM	Through the text	Corrections according to the template
	5. General precautions, 14. Key to symbols used	Information about hazards was rewritten according to the Regulation 1272/2008/EC.
19.09.18 TA	Content	The color of a reagent was specified
08.04.20 KK	Through the text	The text formatting was changed
	Footer	The phrase "Not for use in the Russian Federation" was added
11.03.21 VA	—	The name, address and contact information for Authorized representative in the European Community was changed
31.05.22 KK	1. Intended use	"Indications and contra-indications for use of the reagent kit" subsection was added
	6. Sampling and handling	"Interfering substances and limitations of using test material samples" subsection was added
	13. Quality control	The Authorized representative in the European Community was specified for the contact in case of undesirable effects when using the reagent kit

AmpliSens®

EC REP

Ecoli Dx, s.r.o., Purkyňova 74/2
110 00 Praha 1, Czech Republic
Tel.: +420 325 209 912
Cell: +420 739 802 523

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

Not for use in the Russian Federation