DNA-sorb-B
Nucleic acid Extraction kit

CE IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit	IC	Internal control
	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-B nucleic acid extraction kit is intended for extraction and purification of DNA from the clinical material (whole blood, plasma, urine sediment, saliva, cerebrospinal fluid, sputum, biopsy material, bronchoalveolar lavage, feces).

Indications and contra-indications for use of the reagent kit

Nucleic acid extraction is used in preanalytical stage of in vitro diagnostics by nucleic acid amplification techniques (NAT).

2. PRINCIPLE OF NUCLEIC ACID EXTRACTION

DNA-sorb-B nucleic acid extraction kit is a reagents kit for rapid and efficient manual extraction and purification of DNA from various clinical materials. Lysis solution contains chaotropic agent (guanidine thiocyanate) that lyses cells and denaturates cell proteins. The nucleic acids are then sorbed on silica particles. DNA extracted from clinical samples may be used for PCR diagnostic tests.

3. CONTENT

DNA-sorb-B nucleic acid extraction kit is produced in 1 form:
variant 100, REF K1-2-100-CE.

Variant 100 includes:

Reagent	Description	Volume, ml	Quantity
Lysis Solution	clear liquid from colorless to yellow or pink colour	30	1 vial
Washing Solution 1	colorless clear liquid	30	1 vial
Washing Solution 2	colorless clear liquid	100	1 vial
Universal Sorbent	suspension from white to dark beige colour	1.25	2 tubes
TE-buffer for DNA elution	colorless clear liquid	5.0	2 tubes

Variant 100 is intended for 100 reactions, including controls.

4. ADDITIONAL REQUIRMENTS

- Vacuum aspirator with flask for removing supernatant.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl and 1000 µl).
- Vortex mixer.
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RPM max. 16,000).
- PCR box or Biological cabinet.
- Thermostat for tubes with controlled temperature for 25-100 °C.
- Tube racks.
- Disposable 1.5-ml 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 pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- 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 biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents 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.
- The kit is intended for analysis of specified number of samples (see the section “Content”).
- The kit is ready for use in accordance with the Instruction Manual. Use the kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- 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 in the area where the previous step was performed.

Lysis Solution, 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 alcohol 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.

6. SAMPLING AND HANDLING

NOTE: Obtaining of clinical material samples for PCR-analysis, transportation and storage are described in manufacture’s handbook [2]. It is recommended to read this handbook before starting of the work.

DNA-sorb-B nucleic acid extraction kit is recommended for DNA extraction and purification from: whole blood, blood plasma, urine sediment, saliva, cerebrospinal fluid, sputum, biopsy material, bronchoalveolar lavage, feces.

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

Not for use in the Russian Federation

8. PROTOCOL

8.1. DNA Extraction

Volume of clinical sample for DNA extraction is 0.1 ml.

1. Warm **Lysis Solution** and **Washing Solution 1** (if stored at 2-8 ºC) at the temperature 65 ºC until the crystals disappear.
2. Take the required number of 1.5 ml disposable tubes including one tube for Negative Control of Extraction (Negative Control, C-) and one tube for Positive Control of Extraction (Positive Control, PCE) (if provided with the amplification kit)).
3. Add 10 µl of Internal Control (if it is provided for analysis of this infectious agent) and 300 µl of Lysis Solution to each tube. Mark the tubes.
4. Add 100 µl of a sample to the tube using tips with aerosol filter. Add 100 µl of Negative Control (provided with the amplification kit) to the tube labeled C-. Add 90 µl of Negative Control (provided with the amplification kit) and 10 µl of Positive Control (if it is provided for analysis) to the tube labeled PCE.
5. Thoroughly vortex the tubes then incubate at 65 ºC for 5 min (do not heat the tubes if extracting DNA from blood plasma).
6. Centrifuge all tubes at 5,000 rpm for 5 s. If a sample hasn't dissolved completely, centrifuge the tube at 12,000 rpm for 5 min, transfer the supernatant to a clean tube and use for DNA extraction.
7. Thoroughly resuspend Universal Sorbent on vortex mixer. Into each test tube add 25 µl of Universal Sorbent. Carefully vortex the tubes then leave them in a rack for 2 min. Vortex once again and incubate the tubes for 5 min in a rack.
8. Centrifuge all the tubes at 5,000 rpm for 30 s and carefully remove supernatant without disturbing the pellet using a vacuum aspirator. Use a new tip for each tube.
9. Add 300 µl of Washing Solution 1 to each tube. Vortex thoroughly until sorbent is fully resuspended. Centrifuge at 5,000 rpm for 30 s. Carefully remove supernatant from each tube without disturbing the pellet using vacuum aspirator. Use a new tip for each tube.
10. Add 500 µl of Washing Solution 2 to each tube. Vortex thoroughly until sorbent is fully resuspended. Centrifuge at 10,000 rpm for 30 s. Carefully remove supernatant from each tube using a vacuum aspirator. Use a new tip for every tube.
11. Repeat step 10. Remove supernatant completely.
12. Incubate the tubes with open caps at 65 °C for 5-10 min (to dry the pellet).
13. Add 50 µl of TE-buffer for DNA elution. Vortex the tubes. Incubate the tubes at 65 ºC for 5 min; vortex occasionally while incubating.
14. Centrifuge tubes at 12,000 rpm for 1 min. The supernatant contains purified DNA. The samples are ready for PCR amplification. Be careful not to collect sorbent while removing of the DNA-containing solution. If solution is muddy, centrifuge the tube to precipitate the sorbent.

The purified DNA can be stored:

- at 2–8 °C for 1 week;
- at the temperature not more than minus 16 °C for 1 year.

8.2. Amplification

It is recommended to use AmpliSens® PCR kits.

NOTE: Please carry out the amplification according to the manufacturer instructions.

9. TROUBLESHOOTING

These troubleshooting guides may be helpful in explaining any problem 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 areas.

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

10. TRANSPORTATION

DNA-sorb-B nucleic acid extraction kit should be transported at 2–25.

11. STABILITY AND STORAGE

All components of the **DNA-sorb-B** nucleic acid extraction kit 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.

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-B** 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
		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
27.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
31.03.15 PM	5. General precautions, 14. Key to symbols used	Information about hazards was corrected
28.09.17 PM	Through the text	Correction according to the template
	5. General precautions, 14. Key to symbols used	Information about hazards was rewritten according to the Regulation 1272/2008/EC.
22.12.17 EM	Content	The colour of the reagents was specified
08.04.20 MA	Through the text	The text formatting was changed
	Footer	The phrase "Not for use in the Russian Federation" was added
21.10.20 MM	Footer, 3. Content	The information about variant 50 REF K1-2-50-CE was deleted
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
	5. General precautions	The phrase "for single use" was deleted
	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

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