CE

IVD

For Professional Use Only

DNA-sorb-B

Nucleic acid Extraction kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

DNA-sorb-B is intended for extraction and purification of DNA from clinical materials (whole blood, plasma, urine sediment, saliva, cerebrospinal fluid, sputum, biopsy material, bronchoalveolar lavage, feces).

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 2 forms:

DNA-sorb-B nucleic acid extraction kit variant 50, **REF** K1-2-50-CE;

DNA-sorb-B nucleic acid extraction kit variant 100, **REF** K1-2-100-CE;

DNA-sorb-B nucleic acid extraction kit variant 50 or 100 includes:

Reagent	***Description***	***Variant 50***		***Variant 100***	
		Volume (ml)	***Amount***	***Volume (ml)***	***Amount***
Lysis Solution	colorless clear liquid	15	1 vial	30	1 vial
Washing Solution 1	colorless clear liquid	15	1 vial	30	1 vial
Washing Solution 2	colorless clear liquid	50	1 vial	100	1 vial
Universal Sorbent	white suspension	1.25	1 tube	1.25	2 tubes
TE-buffer for DNA elution	colorless clear liquid	5.0	1 tube	5.0	2 tubes

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

DNA-sorb-B nucleic acid extraction kit variant 100 is intended for 100 reactions, including controls.

4. ADDITIONAL REQUIRMENTS

- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (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 polypropylene sterile 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 and capable of incubating at 25-100 °C.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barrier 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.
- Use disposable gloves, laboratory coats, 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 expiry 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 using a disinfectant such as 0.5 % sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucose membranes. If skin, eyes and mucose membranes 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 move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.

 Xn	**Lysis Solution, Washing Solution 1**	Contains guanidine thiocyonate. Guanidine thiocyonate 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 feeding stuffs;
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 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.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Extraction

Volume of clinical sample for DNA extraction is 0.1 ml.

1. **Lysis Solution** and **Washing Solution 1** (if stored at 2-8 ºC) should be heated to 65 ºC until ice crystals disappear.
2. Collect 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 to each tube **10 µl** of **Internal Control** (if it is provided for analysis of this infectious agent) and **300 µl** of **Lysis Solution**. Label the test tubes.
4. Add **100 µl** of a sample to the tube using tips with aerosol barrier.
5. Prepare Controls as follows:

5.1. Add **100 µl** of **Negative Control** (provided with the amplification kit) to the tube labeled **C-**.

5.2. Add **90 µl** of **Negative Control** (provided with the amplification kit) and **10 µl** of **Positive Control** (provided with the amplification kit) to the tube labeled **PCE**.

6. Vigorously vortex the tubes then incubate at 65 ºC for 5 min in a heating block (do not heat the tubes if extracting DNA from plasma specimens).
7. Centrifuge all tubes at 5,000 r/min for 5 s. If a sample hasn't dissolved completely, spin the tube in a microcentrifuge at 12,000 r/min for 5 min, transfer the supernatant to a clean tube and use for DNA extraction.
8. Thoroughly resuspend **Universal Sorbent** on vortex mixer. Into each test tube add **25 µl** of **Universal Sorbent.** Label the test tubes**.** 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.
9. Centrifuge all tubes at 5,000 r/min for 30 s (for sorbent precipitation) and carefully remove supernatant from each tube without disturbing the pellet using a vacuum aspirator. Use a new tip for every tube.
10. Add **300 µl** of **Washing Solution 1** to each tube. Vortex vigorously until sorbent is fully resuspended. Centrifuge at 5,000 r/min for 30 s. Carefully remove supernatant from each tube without disturbing the pellet using vacuum aspirator. Use a new tip for each tube.
11. Add **500 µl** of **Washing Solution 2** to each tube. Vortex vigorously until sorbent is fully resuspended. Centrifuge at 10,000 r/min for 30 s. Carefully remove supernatant from each tube using a vacuum aspirator. Use a new tip for every tube.
12. Repeat step 11. Remove supernatant completely.
13. Incubate all tubes with caps open at 65 °C for 5-10 min (for sorbent predrying).
14. Add **50 µl** of **TE-buffer for DNA elution**. Vortex vigorously. Incubate the tubes at 65 ºC for 5 min; vortex occasionally while incubating.
15. Centrifuge tubes at 12,000 r/min for 1 min. The supernatant contains purified DNA and is 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 ≤ – 16 °C for 1 year.

If using the DNA samples for a diagnostic assay, follow the instructions supplied by the manufacturer.

8.2. Amplification

Different manufacturers offer PCR amplification kits. We recommend using of AmpliSens® PCR amplification kits.

Please carry out the amplification according to the manufacturer instruction.

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. Use a new sample, store samples appropriately.
- Loss of nucleic acid deposit. Carefully draw off the washing solution and try not to remove the nucleic acid deposit.
- Degradation of the extracted nucleic acid. Plastic free from DNAses and RNAses should be used. Use a new aliquot of kit's component.

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. Use a new aliquot of a component.
- Contamination of the extraction zone by amplicons. Surfaces and instruments should be cleaned using aqueous detergents, wash lab coats. Replace test tubes and tips in use.

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. 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-B

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	EC REP	Authorised representative in the European Community
	Manufacturer	**IC**	Internal control
	Date of manufacture		Harmful
			Flammable

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”