CE

IVD

For Professional Use Only

DNA-sorb-AM

nucleic acid extraction kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

1. INTENDED USE

DNA-sorb-AM nucleic acid extraction kit is intended for extraction and purification of DNA from clinical materials (scrapes and discharges of urogenital tract, throat, rectum, conjunctiva, erosions, ulcers; urine).

2. PRINCIPLE OF NUCLEIC ACID EXTRACTION

DNA-sorb-AM nucleic acid extraction kit is reagents kit for rapid and efficient manual extraction and purification of DNA from various clinical materials. Lysis solution contains chaotropic agent (guanidine chloride) 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-AM nucleic acid extraction kit is produced in 4 forms:

DNA-sorb-AM nucleic acid extraction kit variant 50 (includes controls) REF K1-11-50-CE.

DNA-sorb-AM nucleic acid extraction kit variant 100 (includes controls) REF K1-11-100-CE.

DNA-sorb-AM nucleic acid extraction kit variant 50 (without controls) REF K1-12-50-CE.

DNA-sorb-AM nucleic acid extraction kit variant 100 (without controls) REF K1-12-100-CE.

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

Reagent	***Description***	***Variant 50***		***Variant 100***	
		Volume (ml)	***Quantity***	***Volume (ml)***	***Quantity***
Lysis Solution	colorless clear liquid	15	1 vial	30	1 vial
Washing Buffer	colorless clear liquid	50	1 vial	100	1 vial
Universal Sorbent	white suspension	1.0	1 tube	1.0	2 tubes
TE-buffer for DNA elution	colorless clear liquid	5.0	1 tube	5.0	2 tubes

Additionally provided reagents:

Internal Control complex (ICc)*	colorless clear liquid	1.0	1 tube	1.0	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube	1.0	1 tube
Negative Control (C–)	colorless clear liquid	1.2	1 tube	1.2	1 tube

***** should be used during DNA extraction procedure if followed by PCR-analysis with electrophoretic detection.

****** should be used during DNA extraction procedure if followed by PCR-analysis with hybridization-fluorescent detection.

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

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

4. ADDITIONAL REQUIREMENTS

- 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.
- 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 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.
- 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**	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 Buffer**	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 food 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 samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [2]. It is recommended that this handbook is read before starting work.

DNA-sorb-AM nucleic acid extraction kit is recommended for **DNA** extraction and purification from scrapes and discharges of urogenital tract mucous membranes, throat, rectum, conjunctiva, erosions, ulcers; urine.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Extraction

1. **Lysis Solution** (if stored at 2-8 °C) should be heated to 65 °C until the ice crystals disappear.
2. Collect the required number of 1.5 ml sterile disposable tubes, label them, and place in a tube rack.
3. Centrifuge the tubes with clinical samples at 1500 – 3,000 r/min for 5 sec, then carefully mix using a vortex mixer, and place in a tube rack.
4. Add to each sterile disposable tube **10 µl** of **Internal Control complex (ICc)** (if detection performed by electrophoresis) or **10 µl** of **Internal Control-FL (IC)** (if detection performed by hybridization fluorescent technique) if it is provided for analysis of this infectious agent.

If different detection methods are applied within one test run, it is permitted to use both Internal Controls by adding 10 µl of each.

5. Thoroughly resuspend **Universal Sorbent** on vortex mixer. Into each test tube add **20 µl** of **Universal Sorbent** and **300 µl** of **Lysis Solution** using tips with aerosol barrier.

If the number of processed clinical samples exceeds 50, it is recommended that the whole volume of sorbent and IC are transferred to the tube with Lysis Solution (2 ml of Universal Sorbent and 1 ml of IC per 30 ml of Lysis Solution).Thoroughly stir this suspension and transfer 330 µl of it to the tubes. Prepared mix can be stored at room temperature for up to 2 days. Stir well before use.

6. Add **100 µl** of a sample to the tube using tip with aerosol barrier.
7. Add **100 µl** of **Negative Control** to the tube of Negative Control of extraction (C-).
8. Tightly seal the caps, carefully mix the tubes on vortex mixer, and incubate at 65 °C for 5 min in a heating block. Vortex once again and incubate at room temperature for 2 min.
9. Centrifuge all tubes at 10,000 r/min for 30 sec and carefully remove supernatant from each tube without disturbing the pellet using a vacuum aspirator. Use a new tip (without aerosol barrier) for every tube.
10. Add **1 ml** of **Washing Buffer** into each tube. Vortex vigorously until sorbent is fully resuspended.
11. Repeat step 9.
12. Incubate all tubes with open caps at 65 °C for 5-10 min (for sorbent predrying).
13. Add **100 µl** of **TE-buffer for DNA elution** using tip with aerosol filter. Vortex vigorously until sorbent is fully re-suspended. Incubate tubes at 65 °C for 5 min. Volume of elution can be adjusted up to 150 µl.
14. 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 the DNA-containing solution. 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 minus 16 °C for 1 year.

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

8.2. Amplification

It's recommended to use AmpliSens® PCR kits.

Please carry out the amplification according to the manufacturer's 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. 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. Plastic free from DNAses and RNAses should be used. Use a new aliquot of kit's component.

False positives from extracted 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 using aqueous detergents should be cleaned, 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-AM nucleic acid extraction kit should be transported at 2–8 °C for no longer than 7 days.

11. STABILITY AND STORAGE

All components of the **DNA-sorb-AM** nucleic acid extraction kit (except for Internal Control complex (ICc), Internal Control-FL (IC), Negative Control (C–)) are to be stored at 2-25 °C, 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.

Internal Control complex (ICc), Internal Control-FL (IC) and Negative Control (C–) are to be stored at 2-8 °C. They also must be stable until the expiry date stated on the label.

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-AM 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	**C–**	Negative control of extraction
	Flammable		Harmful

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”