



For *in Vitro* Diagnostic Use

DNA-sorb-AM

nucleic acid extraction kit

Instruction Manual

AmpliSens®



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1. INTENDED USE.

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

2. PRINCIP

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. CONTENTS OF THE KIT.

DNA-sorb-AM nucleic acid extraction kit is produced in 4 forms:

DNA-sorb-AM nucleic acid extraction kit variant 50, [REF](#) K1-11-50-CE, Includes controls for sexually transmitted diseases

DNA-sorb-AM nucleic acid extraction kit variant 100, [REF](#) K1-11-100-CE Includes controls for sexually transmitted diseases

DNA-sorb-AM nucleic acid extraction kit variant 50, [REF](#) K1-12-50-CE Without controls and transport media

DNA-sorb-AM nucleic acid extraction kit variant 100, [REF](#) K1-12-100-CE Without controls and transport media

DNA-sorb-AM 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 fluid	15	1 vial	30	1 vial
Wash Solution	colorless, clear fluid	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 fluid	5.0	1 tube	5.0	2 tubes

Additionally provided reagents:

Reagent	Description	variant 50		variant 100	
		Volume (ml)	Amount	Volume (ml)	Amount
Internal Control complex (IC)*	colorless, clear fluid	1.0	1 tube	1.0	1 tube
Internal Control -FL**	colorless, clear fluid	1.0	1 tube	1.0	1 tube
Negative Control	colorless, clear fluid	1.2	1 tube	1.2	1 tube

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

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

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

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

4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES

- Disposable powder-free gloves
- DNA isolation kit
- Detection agarose kit
- Pipettes (adjustable)
- Sterile pipette tips with aerosol filters (up to 200 µl)
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- PCR box
- Personal thermocyclers
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- Refrigerator for 2–8 °C with deep-freezer with temperature no less then –16°C.
- Reservoir for disposed tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and put the new tip for every procedure.
- Store and handle amplicons separately 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.
- 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.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucose membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- 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.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.



Lysis Solution

Contains guanidine thiocyanate. Guanidine thiocyanate 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 is 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. PROTOCOL

7.1. DNA Isolation

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** (if detection performed by electrophoresis) or **10 µl of Internal Control-FL** (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 10-12 min (for complete sorbent predrying, as residual ethanol can inhibit downstream applications).
13. Add **100 µl of TE-buffer for DNA elution** using tip with aerosol filter. Vortex vigorously until sorbent is fully resuspended. Incubate tubes at 65°C for 5 min. Vortex periodically. 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.

7.2. Amplification.

It's recommended to use AmpliSens® PCR kits.



Please carry out the amplification according to the manufacturer's instructions.

8. 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.

9. STABILITY AND STORAGE.

All components of the **DNA-sorb-AM** nucleic acid extraction kit (except for Internal Control complex, Internal Control-FL, Negative Control) are to be stored between 2°C and 25°C, when not in use. They also must be stable until the expiry date stated on the label.



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

10. 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 State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008

11. QUALITY CONTROL.

In accordance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 –certified Total Quality Management System, each lot of DNA-sorb-AM nucleic acid extraction kit is tested against predetermined specifications to ensure consistent product quality.

12. EXPLANATION OF SYMBOLS.



Manufacturer



Temperature limitation



Use by



Batch code



For *in Vitro* Diagnostic Use



Version



Catalogue number



Internal Control complex



Contains sufficient for <n> tests



Authorized representative in the European Community.



Consult instructions for use



Caution, consult accompanying documents



For working with Rotor-Gene™ 3000/6000



For working with iQ5, iQ iCycler



Positive control



Negative control