DNA-sorb-AM
nucleic acid extraction kit

CE
IVD
For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code	Σ	Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit	IC	Internal control
	Manufacturer	C–	Negative control of extraction
	Date of manufacture		GHS07: Exclamation mark
EC REP	Authorized representative in the European Community		GHS02: Flame
			GHS08: Health hazard

1. INTENDED USE

DNA-sorb-AM nucleic acid extraction kit is intended for DNA extraction from biological material for subsequent testing for the presence of DNA of pathogens which causes sexually transmitted infections by the nucleic acid amplification techniques (NAT):

- discharge (swab, scraping) of the mucous membrane of the urogenital tract (vagina, urethra, cervical canal),
- discharge (swab, scraping) of the mucous membrane of the oropharynx/oral cavity,
- discharge (swab, scraping) of the mucous membrane of the rectum /anal canal,
- discharge of the conjunctiva,
- discharge of vesicular rashes and erosive and ulcerative elements of mucous membranes and skin,
- saliva,
- prostate secretion,
- urine (first portion),
- semen (ejaculate),

and also for DNA extraction from:

- bronchoalveolar lavage fluid/bronchial lavage waters,
- sputum/endotracheal aspirate,
- wound discharge,
- cultures of microorganisms isolated during sowing of human biological material.

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

A test sample is treated by the lysis solution in the presence of the magnetic silica particles (magnetic sorbent). As a result the cell membranes, viral envelopes and other biopolymer complexes are destructed and the nucleic acids are released. The dissolved nucleic acids bind to the sorbent particles while other components of the lysed biological material stay in the solution and are removed by sorbent precipitation at magnetic rack and subsequent washings. The nucleic acids are transferred from the sorbent surface to the solution after adding the buffer for elution to the magnetic sorbent. Then the solution is separated from the sorbent by magnetic force.

The obtained nucleic acid sample is highly purified and free from inhibitors of amplification, which provides high analytical sensitivity of NAT assay.

3. CONTENT

DNA-sorb-AM nucleic acid extraction kit is produced in 2 forms[1]:

variant 100 (includes controls) REF K1-11-100-CE;

variant 100 (without controls) REF K1-12-100-CE.

Variant 100 includes:

Reagent	Description	Volume, ml	Quantity	
			variant 100 (includes controls)	**variant 100** (without controls)
Lysis Solution	colorless clear liquid	30	1 vial	1 vial
Washing Buffer	colorless clear liquid	100	1 vial	1 vial
Universal Sorbent	suspension from white to dark beige colour	1.0	2 tubes	2 tubes
Buffer for elution B	colorless clear liquid	5.0	2 tubes	2 tubes
Internal Control complex (ICc)*	colorless clear liquid	1.0	1 tube	—
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube	—
Negative Control (C–)	colorless clear liquid	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.

Variant 100 is intended for 100 reactions, including controls. The volume of test material is 100 µl.

4. ADDITIONAL REQUIREMENTS

- 1.5-ml disposable polypropylene screwed or tightly closed tubes.
- Screwed caps for tubes.
- Sterile DNase- and RNase-free filter pipette tips (up to 100 µl, 200 µl and 1000 µl).
- Sterile DNase- and RNase-free pipette tips without filter (up to 200 and 1,000 µl).
- Tube racks.
- PCR box.
- Thermostat for tubes with controlled temperature for 25-100 °C.
- Vacuum aspirator with flask for removing supernatant.
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RPM max. 16,000).
- Vortex mixer.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Disposable powder-free gloves and a laboratory coat.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile DNase-and RNase-free pipette tips with aerosol filters and use a 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 the kit if the internal packaging was damaged or its appearance was changed.
- Do not use the kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- 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.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- 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.

[1] The forms are interchangeable: one of the forms can be used instead of the other when performing NAT assay. The use of **variant 100** (without controls) REF K1-12-100-CE instead of **variant 100** (includes controls) REF K1-11-100-CE is possible if controls of extraction are included in the PCR kit used for amplification.

Not for use in the Russian Federation

Lysis Solution  Warning	Contains substance: guanidine hydrochloride H302: Harmful if swallowed. 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+P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents in accordance with national regulations.
Washing Buffer  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 hands 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  Danger	Contains substance: Celite® H350: May cause cancer. H372: Causes damage to organs through prolonged or repeated exposure. P260: Do not breathe dust. P281: Use personal protective equipment as required. P314: Get Medical advice/attention if you feel unwell. P405: Store locked up. P501: Dispose of contents in accordance with national regulations.

6. SAMPLING AND HANDLING

NOTE: See the information about the sampling, conditions of transportation and storage of the test material, as well as the necessity and procedure of its pretreatment before DNA extraction in the *Instruction manual* for the PCR kit.

- **DNA-sorb-AM** nucleic acid extraction kit is recommended for DNA extraction from:
- discharge (swab, scraping) of the mucous membrane of the urogenital tract (vagina, urethra, cervical canal),
- discharge (swab, scraping) of the mucous membrane of the oropharynx/oral cavity,
- discharge (swab, scraping) of the mucous membrane of the rectum /anal canal,
- discharge of the conjunctiva,
- discharge of vesicular rashes and erosive and ulcerative elements of mucous membranes and skin,
- saliva,
- prostate secretion,
- urine (first portion),
- semen (ejaculate),

and also for DNA extraction from:

- bronchoalveolar lavage fluid/bronchial lavage waters,
- sputum/endotracheal aspirate,
- wound discharge,
- cultures of microorganisms isolated during sowing of human biological material.

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.

NOTE: Before carrying out the extraction of nucleic acids, it is necessary to precipitate drops of the test material from the walls of the tube and the inside of the cap by brief centrifugation, then carefully vortex the contents of the tubes, avoiding splashing the material and getting it on the inside of the cap.

Potential interfering substances

Endogenous and/or exogenous substances that may be present in the biological material used for the study were selected to assess potential interference (see Table 1).

Model samples of various biological material without adding and with the addition of potentially interfering substances were tested. The maximum concentration of potential interfering substances in model samples and interference presence are listed in Table 1.

Table 1

Test material	Type of potential interferent	Potential interferent	Tested concentration in a sample	Interference presence
Bronchoalveolar lavage fluid/bronchial lavage waters	Endogenous substances	Mucin	6 mg/ml	Not detected
		Hemoglobin	0.21 g/ml	Not detected
Sputum/endotracheal aspirate	Endogenous substances	Mucin	6 mg/ml	Not detected
Urine (first portion)	Endogenous substances	Albumin	500 mg/ml	Not detected
	Exogenous substances	Azithromycin	1 mg/ml	Not detected
Discharge (swab, scraping) of the mucous membrane of the urogenital tract (vagina, urethra, cervical canal)	Endogenous substances	Mucin	150 µg/ml	Not detected
		Hemoglobin	300 µg/ml	Not detected
Discharge of the conjunctiva	Exogenous substances	Dicaine	0.03 %	Not detected
Prostate secretion	Endogenous substances	Fructose	10 mg/ml	Not detected
	Exogenous substances	Ibuprofen	300 µg/ml	Not detected
Discharge (swab, scraping) of the mucous membrane of the rectum/anal canal	Endogenous substances	Feces	5%	Not detected
	Exogenous substances	Lubricant "Contex Strong"	15%	Not detected
Discharge (swab, scraping) of the mucous membrane of the oropharynx/oral cavity	Exogenous substances	Miramistin®	0.001 %	Not detected
Saliva	Exogenous substances	Chewing tobacco	5 %	Not detected
Cultures of microorganisms isolated during sowing of human biological material	Exogenous substances	Endo medium	10 µl	Not detected
		UriSelect medium	10 µl	Not detected
Discharge of vesicular rashes and erosive and ulcerative elements of mucous membranes and skin	Exogenous substances	Acyclovir	0.1 µg	Not detected
Wound discharge	Endogenous substances	Hemoglobin	0.21 g/ml	Not detected
	Exogenous substances	An aqueous solution of chlorhexidine bigluconate	2.5%	Not detected
Semen (ejaculate)	Endogenous substances	Fructose	10 mg/ml	Not detected
	Exogenous substances	Ibuprofen	300 µg/ml	Not detected

7. WORKING CONDITIONS

DNA-sorb-AM nucleic acid extraction kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. DNA Extraction

1. Warm **Lysis Solution** (if stored at 2–8 °C) at the temperature 65 °C until the crystals disappear.
2. Take the required number of 1.5-ml disposable tubes (including controls if they are provided for analysis). Mark the tubes.
3. 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 PCR-analysis).

NOTE: It is allowed to change the volume of Internal Control according to the *Instruction manual* for the used PCR kit[2].

4. Thoroughly resuspend **Universal Sorbent** on vortex mixer. Into each test tube add **20 µl** of resuspended **Universal Sorbent** and **300 µl of Lysis Solution** using filtered tips.

NOTE: If there is large amount of test samples, it is recommended to transfer the whole volume of **Universal sorbent** and **Internal Control** (if it is provided) to the tube with **Lysis Solution** (2 ml of **Universal Sorbent** and 1 ml of **Internal Control** per 30 ml of **Lysis Solution**).Thoroughly stir obtained 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.

5. Add **100 µl** of test samples to the tube containing **Lysis Solution**, **Universal sorbent** and **Internal Control** (if it is used) using filtered tip. Mix by pipetting, close the caps tightly.

NOTE: It is allowed to change the volume/quantity of test sample according to the Instruction manual for the used PCR kit (for example, cell sediment or bacterial cells from a dense nutrient medium).

6. Add **100 µl** of **Negative Control (C–)** to the tube of Negative Control of extraction (C-), add **90 µl of Negative Control (C–)** and **10 µl Positive Control** (if they are provided for the PCR-analysis).

NOTE: It is allowed to change the volume and dilution of **Negative Control (C–)** and **Positive Control** according to the Instruction manual for the used PCR kit.

7. Tightly seal the caps, carefully mix the tubes on vortex mixer, and incubate at 65 °C for 5 min. Vortex once again and incubate at room temperature for 2 min.
8. Centrifuge all tubes at 10.000 rpm for 30 s and carefully remove supernatant from each tube without disturbing the pellet using a vacuum aspirator. Use a new tip (without filter) for every tube.
9. Add **1 ml** of **Washing Buffer** into each tube. Vortex thoroughly until **Universal sorbent** is fully re-suspended.
10. Repeat step 8.
11. Incubate all tubes with open caps at 65 °C for 5-10 min (to dry the **Universal sorbent**).
12. Add **100 µl** of **Buffer for elution B** using filter tip. Vortex thoroughly until sorbent is fully re-suspended.

NOTE: It is allowed to change the elution volume according to the Instruction manual for the used PCR kit.

13. Incubate tubes at 65 °C for 5 min.
14. Centrifuge tubes at 12.000 rpm for 1 min. The supernatant contains purified DNA.

The purified DNA could be stored:

– at 2-8 °C for 1 week;
– at the temperature from minus 24 °C to 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.

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

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 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

[2] If different detection methods are applied within one test run, it is permitted to use both Internal Controls (**Internal Control complex (ICc)** and **Internal Control-FL (IC)**) by adding 10 µl of each.

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 questions or if you 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. It is allowed to transport the reagent kit at 2–26 °C for no longer than 5 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. All components of the **DNA-sorb-AM** nucleic acid extraction kit arestable 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: Internal Control complex (ICc), Internal Control-FL (IC) and Negative Control (C–) are to be stored at 2-8 °C.

12. REFERENCES

1. H. Zetzsche, H.-P. Klenk, M.J. Raupach, T. Knebelsberger, B. Gemeinholzer. Comparison of methods and protocols for routine DNA extraction in the DNA Bank Network // DNA Bank Network. URL: https://www.dnabank-network.org. [Date of request: 05.02.2020]
2. Breadmore M.C., Wolfe K.A., Arcibal I.G., et al. Microchip-Based Purification of DNA from Biological Samples // Anal. Chem. 2003. V. 75. P. 18801886.

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-AM** 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
25.09.17 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.
16.01.18 ME	Content	The color of the reagents was specified
06.03.18 PM	Footer, Content	REF K1-12-50-CE was deleted
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 K1-11-50-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
14.12.23 EM	5. General precautions	Information about hazards was rewritten according to the Regulation (EU) 2020/878
28.04.25 HM	1. Intended use	The intended use was specified. The list of biological material was expanded
	2. Principle of nucleic acid extraction	Section was rewritten
	3. Content	For **Lysis Solution** description was changed. **TE-buffer for DNA elution** was renamed to **Buffer for elution B**. The information about the interchangeability of reagent kit forms was added
	4. Additional requirements	The list of additional requirements was specified.
	5. General precautions	List of precautions was expanded
	6. Sampling and handling	List of potentially interfering substances was added
	7. Working conditions	Temperature range was changed. Relative humidity was added
	8. Protocol	Working procedure was rewritten
	10. Transportation	Transportation diapason was changed
17.07.25 PM	3. Content	The information in the table has been clarified for each form

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