AmpliSens® DNA-sorb-D
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	C–	Negative Control of extraction
	Manufacturer	PCE	Positive Control of extraction
	Date of manufacture		GHS07: Exclamation mark
EC REP	Authorized representative in the European Community		GHS02: Flame
			GHS05: Corrosion
			GHS08: Health hazard

1. INTENDED USE

AmpliSens® DNA-sorb-D nucleic acid extraction kit is intended for extraction of DNA from epithelial cells (cervical swabs) taken into the transport medium for liquid-based cytology (for example, PreservCyt (Hologic Inc., USA)) for subsequent analysis by the polymerase chain reaction (PCR).

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

Test samples are treated using mucolysin to dissolve cervical mucus. Then the cells are washed out off the transport medium using phosphate buffer solution (PBS-buffer) and cells membranes are destructed by lysis with detergent (cytolysin). As a result the nucleic acids is released and the proteins are degradated by protease. DNA without proteins is purified by sorption on silica gel. 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 centrifugal sedimentation and subsequent washings. The nucleic acids are transferred from the silica surface to the solution after adding the buffer for elution to the sorbent. Then the solution is separated from the sorbent by centrifugation.
The obtained nucleic acid sample is highly purified and free from inhibitors of amplification, which provides high analytical sensitivity of PCR assay.

3. CONTENT

AmpliSens® DNA-sorb-D nucleic acid extraction kit is produced in 1 form:
variant 100, REF K8-2331-100-CE.

Variant 100 includes:

Reagent	Description	Volume, ml	Quantity
Cytolysin	colorless clear liquid	5.0	2 vials
Mucolysin	colorless clear liquid	100	1 vial
PBS-buffer	colorless clear liquid	100	1 vial
Lysis Solution[1]	clear liquid from colorless to yellow or pink colour	30	1 vial
Washing Buffer	colorless clear liquid	100	1 vial
Universal Sorbent	suspension from white to dark beige colour	1.0	3 tubes
Buffer for elution B	colorless clear liquid	5.0	2 tubes
Negative Control (C-)	colorless clear liquid	1.2	2 tubes

Variant 100 is intended for 100 reactions, including controls.

4. ADDITIONAL REQUIRMENTS

- Transport medium for liquid-based cytology (PreservCyt (Hologic Inc., USA)).
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile DNase- and RNase-free pipette tips with aerosol filters (from 200 to 1.000 µl).
- Sterile DNase- and RNase-free pipette tips without aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RCF max. 12,000 x g).
- PCR box.
- Vacuum aspirator with flask for removing supernatant.
- 1.5 ml and 5-ml disposable polypropylene sterile tubes with screw or tight-fitting caps.
- Thermostat for tubes with controlled temperature and capable of incubating at 25-100 °C.
- 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 DNase- RNase-free pipette filter tips and use 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 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 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 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.
- Use of this product should be limited to personnel trained in the techniques of DNA/RNA extraction.
- 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 to the area where the previous step was performed.

Lysis Solution 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 Buffer Warning	Contains substance: Isopropanol 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.
Mucolysin Warning	Contains substance: 2-Mercaptoethanol H317: May cause an allergic skin reaction P261: Avoid breathing dust/fume/gas/mist/vapours/spray. P280: Wear protective gloves/protective clothing/eye protection/face protection. P302 + P352: IF ON SKIN: Wash with plenty of water P333 + P313: If skin irritation or a rash occurs: Get medical advice. P363: Wash contaminated clothing before reuse. P501: Dispose of contents in accordance with national regulations.

[1] If **Lysis Solution** is stored at the temperature 2- 8 °C crystalline precipitate may form.

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.

AmpliSens® DNA-sorb-D nucleic acid extraction kit is intended for DNA extraction from the biological material (cervical swabs taken into the transport medium for liquid-based cytology).
The samples can be stored in the transport medium for liquid-based cytology at the temperature from 18 to 25 °C – for 1 year.
The volume of the sample for pretreatment is 2-5 ml.
The volume of test sample (pretreated) for extraction is 100 µl.

Pretreatment

NOTE: Take an aliquot of cells for the PCR-analysis using only disposable filter tips and disposable tube. It is important to take first an aliquot of cells for the PCR-analysis and then for the liquid-based cytology.

Epithelial cells concentrating

Method 1

1. Take the required number of disposable 5-ml tubes equal to the number of test sample. Mark the tubes. Shake intensively each vial with the sample for liquid-based cytology for cells disintegration. Then gently open and transfer 2-5 ml of cells (depending on density of cells suspension) into the prepared tubes.
2. Leave the tubes in the rack at the temperature from 18 to 25 °C for the night for cells sedimentation and centrifuge on microcentrifuge for **10 min** at **600 g** (for example, **3,000 rpm** for microcentrifuge MiniSpin, Eppendorf Manufacturing Corporation).
3. Remove the supernatant from each tube. Do not disturb the cell pellet. Use a new one 1000-µl filter tip for each sample and pipette.
4. Transfer gently the rest of the cell pellet with supernatant (~1 ml) into a new one 1.5-ml tube using a new one filter tip for each sample. Mark the tubes and centrifuge at **10,000 g** (for example, **12,000 rpm** for microcentrifuge MiniSpin, Eppendorf Manufacturing Corporation) for **2 min**.
5. Remove the supernatant from each tube. Do not disturb the cell pellet. Use a new one 200-µl filter tip for each sample and vacuum aspirator. Leave **100-200 µl of pellet** with supernatant.

Method 2

1. Shake intensively each vial with the sample for liquid-based cytology for cells disintegration and leave for the night for cells sedimentation.
2. Transfer 0.5-1.0 µl of cells from the bottom of the vial into a new one 1.5-ml tube using 1000-µl tip and pipette. Mark the tube.
3. Centrifuge at **10,000 g** (for example, **12,000 rpm** for microcentrifuge MiniSpin, Eppendorf Manufacturing Corporation) for **2 min**.
4. Remove the supernatant from each tube. Do not disturb the cell pellet. Use a new one 200-µl filter tip for each sample and vacuum aspirator. Leave **100-200 µl of pellet** with supernatant.

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

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

8. PROTOCOL

8.1 Cells washing

1. Add **1 µl of Mucolysin** to the tube with the pellet, thoroughly mix by vortexing and leave it in the tube rack for **30 min.** If there are traces of undissolved mucus plug in the tube, vortex the tube and leave it in the tube rack for another **10-15 min.**
2. Centrifuge the tubes at **10,000 g** for **2 min**.
3. Carefully remove the supernatant inserting the tip near the internal tube wall and using vacuum aspirator and pipette tips without filter. Take a new tip for each sample. Leave **100-200 µl** of the pellet.
4. Add to the pellet **1 µl** of **PBS-buffer**, thoroughly mix by vortexing.
5. Centrifuge the tubes at **10,000 g** for **2 min**.
6. Carefully remove the supernatant inserting the tip near the internal tube wall and using vacuum aspirator and pipette tips without filter. Take a new tip for each sample. Leave **100-200 µl** of the pellet. Then carefully remove the rest of the supernatant using the **pipette** with a new tip for each sample.
7. Add **100 µl** of **Cytolysin** to the cells pellet, thoroughly resuspend by pipetting using a new tip for each sample. Tightly close and vortex the tubes.
8. Incubate the tubes at **60 °C** for **2 hours** or **one night**.

8.2 DNA purification

1. Warm up **Lysis Solution** (if it was stored at 2-8 °C) at **65 °C** until crystals disappear.
2. Prepare the tubes for the Negative Control of Extraction (C–) and the Positive Control of Extraction (PCE) (if provided for PCR assay)[2]. Add **100 µl** of **Negative Control**[3] reagent to the tube labeled C– (Negative control of Extraction). Add **90 µl** of **Negative Control**[3] reagent and **10 µl** of the **Positive Control**[3] reagent to the tube labeled PCE (Positive control of Extraction).
3. Add **300 µl** of **Lysis Solution** into the each tube (including the tubes with the Negative Control of Extraction (C–) and the Positive Control of Extraction (PCE)).
4. Tightly close the tubes, mix by vortexing and warm them at **65 °C** for **5 min**. Sediment the drops from walls of tubes by vortexing. If the sample has not been completely dissolved, centrifuge the tube at **10,000 g** for **5 min.** Transfer the supernatant into the new 1.5-ml tube and use it for DNA extraction.
5. Thoroughly resuspend **Universal Sorbent** on vortex mixer. Add **25 µl** of **Universal Sorbent** into the each tube. Vortex the tubes then leave them in a tube rack for **2 min**. Vortex the tubes once again and leave them for **5 min** in the tube rack.
6. Centrifuge the tubes at **2,000 g** for **30 s** (for example, at 5,000 rpm for MiniSpin, Eppendorf Manufacturing Corporation).
7. Carefully remove the supernatant inserting the tip near the internal tube wall and using vacuum aspirator with new tips without filters for each tube.
8. Add **1 ml** of **Washing Buffer**, tightly close the tubes, mix on vortex until complete sorbent resuspension.
9. Centrifuge the tubes at **2,000 g** for **30 s**
10. Carefully remove the supernatant inserting the tip near the internal tube wall and using vacuum aspirator with new tips without filters for each tube.
11. Incubate the tubes with open caps at **65 ºC** for **5–10 min** (for sorbent drying).
12. Add **100 µl** of **Buffer for elution B** into the tubes. Tightly close the tubes and vortex them until complete sorbent resuspension. Incubate the tubes at **65 ºC** for **5 min,** vortex occasionally while incubating. **It is allowed to increase the volume of elution up to 150 µl (see the *Instruction Manual* enclosed to the PCR kit).**
13. Centrifuge tubes at **10,000 g** for **1 min**. The supernatant contains purified DNA and is ready for PCR amplification.

The purified DNA can be stored after transferring supernatant to the sterile tubes:
- at 2–8 °C for 1 week;
- at the temperature from minus 24 to minus 16 °C for 1 year.

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's necessary to use a new sample, store samples appropriately.
- Loss of nucleic acid pellet. Carefully draw off the washing solution and try not to remove the sorbent.
- Degradation of the extracted nucleic acid. It's necessary to use plastic free from DNases and RNases.

False positives with extraction product:

- Contamination during sample extraction. It's 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's necessary to repeat the test.
- Contamination of the Extraction Area by amplicons. It's necessary to clean surfaces and instruments using aqueous detergents, wash lab coats, replace test tubes and tips in use. Use different laboratory coats in Extraction, Amplification and Detection areas.

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

10. TRANSPORTATION

AmpliSens® DNA-sorb-D nucleic acid extraction kit should be transported at 2–25 °C.

11. STABILITY AND STORAGE

All components of the **AmpliSens® DNA-sorb-D** nucleic acid extraction kit are to be stored at 2-8 ºC, when not in use. All components of the **AmpliSens® DNA-sorb-D** nucleic acid extraction kit are 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. 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 **AmpliSens® DNA-sorb-D** 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
23.03.20 EM	Stability and Storage	The storage temperature range was changed from 2-25 ºC to 2-8 ºC
08.04.20 MA	Through the text	The text formatting was changed
	Footer	The phrase "Not for use in the Russian Federation" was added
25.03.21 KK	—	The name, address and contact information for Authorized representative in the European Community was changed
31.05.22 EM	1. Intended use	"Indications and contra-indications for use of the reagent kit" subsection was added
	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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[2] The results of carrying out the control extraction reactions are used for validity evaluation of the PCR assay of biological samples. Analysis of the results for controls is performed according to the *Instruction Manual* enclosed to the PCR kit.
[3] It is allowed to change the volume of the control samples (see the *Instruction Manual* enclosed to the PCP kit).