AmpliSens® MAGNO-sorb-URO
nucleic acid extraction kit

CE
IVD
For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Contains sufficient for <n> tests
LOT	Batch code		Use-by Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version	C–	Negative Control of extraction
	Temperature limit	PCE	Positive Control of extraction
	Manufacturer	IC	Internal Control
	Date of manufacture		GHS02: Flame
EC REP	Authorized representative in the European Community		GHS05: Corrosion
	Caution		GHS07: Exclamation mark

1. INTENDED USE

AmpliSens® MAGNO-sorb-URO nucleic acid extraction kit is intended for extraction of DNA from the biological material for subsequent testing for the presence of DNA of pathogens which causes sexually transmitted infections, other infections of reproductive organs and urinary tract by the nucleic acid amplification techniques (NAT):

- vaginal discharge (swab, scrape),
- epithelial scrapes from cervical canal taken into **Transport medium with mucolytic agent**,
- epithelial scrapes from cervical canal taken into the transport alcohol medium for liquid-based cytology,
- epithelial scrapes from urethra,
- oropharyngeal discharge (swab, scrape),
- discharge (scrape/swab) from rectum/anal canal,
- urine.

Indications and contra-indications for use of the reagent kit
DNA extraction is used in preanalytical stage of in vitro diagnostics by NAT.

2. PRINCIPLE OF NUCLEIC ACID EXTRACTION

A test sample[1] 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 DNA is released. The dissolved DNA binds to the sorbent particles while other components of the lysed biological material stay in the solution and are removed by sorbent sedimentation at a magnetic rack and subsequent washings. The DNA is 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 DNA sample is highly purified and free from inhibitors of amplification that ensures high analytical sensitivity of NAT assay.

3. CONTENT

AmpliSens® MAGNO-sorb-URO nucleic acid extraction kit is produced in 2 forms:
variant 100, REF K4-2181-100-CE
variant 100D, REF K4-2182-100-CE

Variant 100 includes:

Reagent	Description	Volume, ml	Quantity
Lysis Solution MS-URO	clear liquid from colorless to yellow or pink colour[2]	45	1 vial
Component A-1	colorless clear liquid	1.0	1 tube
Washing Solution 4	colorless clear liquid	20	1 vial
Magnetized silica	suspension of magnetic particles	1.0	1 tube
Buffer for elution B	colorless clear liquid	15	1 vial
Internal Control-FL (IC)	colorless clear liquid	1.0	1 tube
Negative Control (C–)	colorless clear liquid	1.2	2 tubes

Variant 100 is intended for DNA extraction from 100 samples (including controls).

Variant 100D includes:

Reagent	Description	Volume, ml	Quantity
Lysis Solution MS-URO	clear liquid from colorless to yellow or pink colour[2]	45	1 vial
Component A-1	colorless clear liquid	1.0	1 tube
Washing Solution 4	colorless clear liquid	20	1 vial
Magnetized silica	suspension of magnetic particles	1.0	1 tube
Buffer for elution B	colorless clear liquid	15	1 vial
Mucolysin	colorless clear liquid	100	1 vial
PBS-buffer	colorless clear liquid	100	1 vial
Cytolysin	colorless clear liquid	10	1 vial
Negative Control (C–)	colorless clear liquid	1.2	2 tubes

Variant 100D is intended for DNA extraction from 100 samples (including controls).

4. ADDITIONAL REQUIREMENTS

- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette filter tips (up to 200 and 1,000 µl).
- Sterile pipette tips (up to 200 µl).
- 1.5-ml disposable polypropylene screwing or tightly closed tubes.
- 2-ml disposable polypropylene screwing or tightly closed tubes - when using **variant 100D**.
- Tube racks.
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RCF min. 12,000 x g) - when using **variant 100D**.
- Vortex mixer.
- Thermostat for Eppendorf tubes with capable of incubating at 25-100 °C.
- Magnetic racks for Eppendorf 1.5-ml tubes.
- Vacuum aspirator with flask for removing supernatant.
- PCR box or biological cabinet.
- Refrigerator with the temperature from 2 to 8 °C.
- Reservoir for used tips.

In case of using automated station for nucleic acid extraction:

- Open automated DNA extraction systems with magnetic stirring (for example, Auto-Pure 96 (Hangzhou Allsheng Instruments Co.,Ltd., China), KingFisher Flex (Thermo FS (Termo Fisher Scientific), Finland)).
- Open automated DNA extraction pipetting systems (for example, NEON 100 (Xiril AG, Switzerland), MicroLab STARlet (Hamilton Bonaduz AG, Switzerland)).
- Set of consumables for used automated system according to the manufacturer's recommendations including consumables for stations with magnetic stirring:
  - 96-tip comb;
  - 96-deepwell plate;
  - 96-elution plate;
  - sterile self-adhesive film for plates.
- 1.5-5 ml disposable polypropylene or polysterol tubes with caps – to prepare the required volume of a mixture of magnetized silica.
- Sterile pipette filter tips (up to 100 µl).
- Sterile pipette filter tips (up to 1000 µl).
- Single-channel pipettes (adjustable).
- 8-channel pipettes.
- Reservoirs (baths) for filling 8-channel pipettes.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette filter tips[3] and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- 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 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.
- 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.

[1] The material transferring into a transport media and/or pretreatment is required for some types of the biological material.
[2] If **Lysis Solution MS-URO** is stored below 20 °C, crystalline precipitate may form.
[3] Use disposable tips without filters for removing the supernatant during the extraction process.

- 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 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.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

Lysis Solution MS-URO  Danger	Contains substance: isopropanol, guanidine thiocyanate, guanidine hydrochloride, Triton X-100. H226: Flammable liquid and vapour. H314: Causes severe skin burns and eye damage. H318: Causes irreversible effects in case of contact with eyes H336: May cause drowsiness or dizziness EUH032: Contact with acids liberates very toxic gas. P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P260: Do not breathe vapours. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. P501: Dispose of contents in accordance with national regulation.
Washing Solution 4  **Danger**	Isopropanol EC No 200-661-7 CAS No 67-63-0 H225: Highly 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.
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.

6. SAMPLING AND HANDLING

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

AmpliSens® MAGNO-sorb-URO nucleic acid extraction kit is recommended for DNA extraction from:

- vaginal discharge (swab, scrape),
- epithelial scrapes from cervical canal taken into **Transport medium with mucolytic agent**,
- epithelial scrapes from cervical canal taken into the transport alcohol medium for liquid-based cytology,
- epithelial scrapes from urethra,
- oropharyngeal discharge (swab, scrape),
- discharge (scrape/swab) from rectum/anal canal,
- urine.

Interfering substances and limitations of using test material samples

The information about limitations of using test material samples is specified in the Instruction Manual of the PCR kit.

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 potentially 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
Oropharyngeal discharge (swab, scrape)	Endogenous substances	Mucin	5%	Not detected
	Exogenous substances	Lugol's solution with glycerin	0,5%	Not detected
		Chlorhexidine bigluconate aqueous solution	2.5%	Not detected
Discharge (scrape/swab) from rectum/anal canal	Exogenous substances	Whole blood	40%	Not detected
		Fecal fats	40%	Not detected
		Mucin (mucus)	3%	Not detected
	Endogenous substances	“Loperamide-Akrikhin”	5 mg/ml	Not detected
		“Hydrocortisone”	3% (the drug weight to the volume of the test sample)	Not detected
Urine	Endogenous substances	Albumin	500 mg/l	Not detected
	Exogenous substances	“Azithromycin”	0.8 mg/ml	Not detected
Vaginal discharge (swab, scrape), Epithelial scrapes from cervical canal taken into the transport alcohol medium for liquid-based cytology, Epithelial scrapes from cervical canal taken into **Transport medium with mucolytic agent**, Epithelial scrapes from urethra	Endogenous substances	Hemoglobin	260 µg/ml	Not detected
		Lactoferrin	5 µg/ml	
		Glycogen	120 mg/ml	
		Mucin	150 µg/ml	
	Exogenous substances	“Miramistin®”	16 % (the drug volume to the volume of the test sample)	
		“Clotrimazole”		
		“Metronidazole”		
		neomycin + nystatin+ polymyxin B (“Polygynax”)		
		nystatin + nifuratel (“Macmiror® Complex”)		
		“Chlorhexidine bigluconate” aqueous solution		
		“Hasico For Women”, gel lubricant		
		“Contex Silk”, silicone gel lubricant for intimate use		
		“Play Feel”, gel lubricant for sensitivity increasing		

7. WORKING CONDITIONS

AmpliSens® MAGNO-sorb-URO 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 Variant 100

The volume of test sample for the extraction is 100 µl.
Test material:

- vaginal discharge (swab, scrape),
- epithelial scrapes from cervical canal taken into **Transport medium with mucolytic agent**,
- epithelial scrapes from urethra,
- oropharyngeal discharge (swab, scrape),
- discharge (scrape/swab) from rectum/anal canal,
- urine.

NOTE: See the information about pretreatment of biological material the Instruction manual for the used PCR kit

NOTE: **Lysis Solution MS-URO** has unpleasant odor. Work in the PCR box

Manual procedure in case of using magnetic racks

1. If there is a precipitate in **Lysis Solution MS-URO** warm it up at 60 °C until crystals disappear. Resuspend **Magnetized silica** on vortex.
2. Prepare the required number of disposable 1.5-ml tubes (including tubes for the negative and positive controls of extraction, if they are provided for analysis). Mark the tubes.
3. Mix in a separate tube **Internal Control-FL (IC)** (if it is provided for analysis), **Component A-1**, and **Magnetized silica** in the following proportion calculated per one sample: **10 µl** of **Internal Control-FL (IC)**, **10 µl** of **Component A-1**, and **10 µl** of **Magnetized silica**. Prepare the mixture for total number of test and control samples plus extra sample. For example:

Number of samples for DNA extraction	Internal Control-FL (IC), µl	Component A-1, µl	Magnetized silica, µl
6	70	70	70
12	130	130	130
18	190	190	190
24	250	250	250

4. Add **30 µl** of the resuspended mixture of **Internal Control-FL (IC)**, **Component A-1**, and **Magnetized silica** into each tube (or **20 µl** of the mixture in case of exogenous IC absence).
5. Add **450 µl** of **Lysis Solution MS-URO** into the tubes.
6. Add **100 µl** of **test sample** into each tube and mix by vortexing.

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

7. Add **100 µl** of **Negative Control (C–)** reagent into the tube for the Negative Control of extraction (C–) and **100 µl** of **required Positive Control sample** into the tube for the Positive Control of extraction (PCE) (if they are provided for PCR). Tightly close the tubes and thoroughly mix by vortexing.

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.

8. Incubate the tubes at **60 °C** for **10 min** in a thermostat.
9. Mix the contents of the tubes, then sediment the drops by short centrifugation. Transfer the tubes to a magnetic rack, and incubate for **2 min**.

NOTE: If it is inconvenient/impossible to open the tubes in a magnetic rack without sorbent resuspension, it is necessary to open the tubes before placing them in a magnetic rack.

10. Leaving the tubes in the magnetic rack, carefully remove the supernatant inserting the tip near the internal tube wall and using vacuum aspirator and a new tip for each sample.
11. Add **200 µl** of **Washing Solution 4** to the tubes. Tightly cap the tubes.

NOTE: If it is inconvenient/impossible to tightly close the tubes in a magnetic rack it is necessary to place the tubes in a regular tube rack.

12. Resuspend **Magnetized silica** by mixing on vortex. Then sediment the drops by short centrifugation.
13. Transfer the tubes to a magnetic rack for **1 min**.
14. Open the tubes, then carefully remove the supernatant according to the item 10.
15. Dry the magnetized silica. To do this, open the tubes and incubate them in a thermostat at **60°C** for **5 min**. In case of incomplete drying of the sorbent, it is allowed to increase the time up to **10 min**.
16. Add **100 µl** of **Buffer for elution B** into each tube, close the tubes and mix on vortex.

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

17. Transfer the tubes into a thermostat at **60 °C** for **5 min,** mix by vortexing after **2 min**.
18. Sediment the drops by short centrifugation and transfer the tubes to a magnetic rack. Incubate for **2 min**. Supernatant contains purified DNA.

NOTE: Do not take the tubes away from a magnetic rack when removing DNA.

Procedure in case of using automated station for nucleic acid extraction

The detailed information work with automated stations see in the *Instruction Manual* given by the manufacturer of the instrument.

Extraction using stations with magnetic stirring (for example, Auto-Pure 96 (Hangzhou Allsheng Instruments Co.,Ltd., China), KingFisher Flex (Thermo FS, Finland)).
Only the protocols located on the amplisens.ru website should be used for extraction.

NOTE: The extraction protocol for the automated station contains specific parameters that are not available for manual extraction.

1. If there is a precipitate in **Lysis Solution MS-URO** warm it up at 60 °C until crystals disappear. Resuspend **Magnetized silica** on vortex.
2. Prepare the required number of 96-well plates (see point 3) compatible with the instrument; mark them according to the protocol.
3. Add in the plates according to the marking and quantity of samples for extraction:
   - **450 µl** of **Lysis Solution MS-URO** per deep well plate,
   - **200 µl** of **Washing Solution 4** per deep well plate,
   - **100 µl** of **Buffer for elution B** per the plate for elution.

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

4. Mix in a separate tube in the following proportion calculated per one sample:
   - **10 µl** of **Internal Control-FL (IC)** (if it is provided for analysis),
   - **10 µl** of **Component A-1**,
   - **10 µl** of **Magnetized silica**.

Prepare the mixture for total number of test and control samples plus extra samples (for example, in case of extraction from 96 samples it is recommended to prepare mixture for 100 extractions, i.e. plus 4 extra samples).

5. Add **30 µl** of thoroughly resuspended mixture (or **20 µl** in case of exogenous Internal control absence) to each well with **Lysis Solution MS-URO**.
6. Add **100 µl of test and control (**if they are provided for analysis**) samples** in each well with **Lysis Solution MS-URO** and mixture according to the marking.
7. Choose the protocol of extraction, place the prepared plates and tip-plate according to the protocol and placement indicators.
8. Run the DNA extraction protocol.
9. If necessary, seal the elution plate with the purified DNA using a self-adhesive film at the end of the extraction.

Extraction using pipetting stations (for example, NEON 100 (Xiril AG, Switzerland), MicroLab STARlet (Hamilton Bonaduz AG, Switzerland)).

NOTE: Extraction using pipetting stations is carried out only for vaginal discharge (swab, scrape), epithelial scrapes from cervical canal taken into **Transport medium with mucolytic agent**; epithelial scrapes from urethra, urine.

1. Arrange the required number of the consumables on the desktop of the station: tips, test tubes/plates for elution.
2. If there is a precipitate in **Lysis Solution MS-URO** warm it up at 60 °C until crystals disappear. Resuspend **Magnetized silica** on vortex.
3. Place the tubes with Internal Control (if it is provided for analysis), **Component A-1** and **Magnetized silica** on the desktop of the station.
4. Pour **Lysis Solution MS-URO**, **Washing Solution 4**, and **Buffer for elution B** into the appropriate containers placed on the desktop of the station.
5. Place the tubes with test samples on the desktop of the station. Choose the rack according to the type of used tubes.
6. Add no less than **150 µl** of **Negative Control (C–)** into the tube for the Negative Control of extraction (C–) and no less than **150 µl** of **required Positive Control sample** into the tube for the Positive Control of extraction (PCE) (if they are provided for PCR).

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. The volume of control samples should be increased taking into account extra samples.

7. Choose and run the protocol of extraction.
8. If the elution was carried out into a plate, seal the elution plate with the purified DNA using a self-adhesive film at the end of the extraction if necessary.

Extracted DNA can be stored:
- at 2–8 °C for 1 week,
- at the temperature from minus 24 to minus16 °C for 1 year.

In case of manual extraction transfer the supernatant for purified DNA storage into a new tube without disturbing the pellet.
In case of automatic extraction transfer eluate into the tubes for purified DNA storage at subzero temperature.

8.2 Variant 100

The volume of test sample for the extraction is about 100 µl of lysed cellular pellet.
Test material:

- epithelial scrapes from cervical canal taken into the transport alcohol medium for liquid-based cytology.

NOTE: Take an aliquot of cells for the nucleic acid amplification techniques (NAT) using only disposable filter tips into the disposable tube. It is important to take first an aliquot of cells for the NAT and then for the liquid-based cytology.

A. Epithelial cells concentrating

Method 1:

1. Shake intensively each vial with the sample for liquid-based cytology for cells disintegration and leave at a temperature **from 18 to 25 °C for 30 minutes** for cell sedimentation.
2. Take 1 ml of the sediment with the automatic pipettes and carefully transfer into new 1.5-ml tubes.
3. Centrifuge the tubes in a microcentrifuge **for 5 min at 12,000 g**.
4. Remove the supernatant from each tube. Do not disturb the pellet. Use a vacuum aspirator and a new one 200-µl filter tip for each sample. Leave no more than **100 µl of pellet** with supernatant.

Method 2

1. Shake intensively each vial with the sample for liquid-based cytology for cells disintegration.
2. Immediately withdraw **1.0-2.0 ml** of the cell suspension with an automatic dispenser and transfer into new 2.0-ml tubes. Mark the tubes.
3. Centrifuge the tubes in a microcentrifuge **for 5 min at 12,000 g**.
4. Remove the supernatant from each tube. Do not disturb the pellet. Use a vacuum aspirator and a new one 200-µl filter tip for each sample. Leave not more than **100 µl of pellet** with supernatant.

B. Washing and lysis of epithelial cells

If there is mucus in the samples of concentrated epithelial cells, it is necessary to treat them with **Mucolysin** reagent.
Start the preparation of concentrated epithelial cells by washing with PBS buffer (point 4) if there is no mucus accumulation in the samples.

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 **1 hour**.

C. DNA purification using magnetized silica

NOTE: **Lysis Solution MS-URO** has unpleasant odor. Work in the PCR box.

Manual procedure in case of using magnetic racks

1. If there is a precipitate in **Lysis Solution MS-URO** warm it up at 60 °C until crystals disappear. Resuspend **Magnetized silica** on vortex.
2. Mix in a separate tube **Internal Control** (if it is provided for PCR), **Component A-1**, and **Magnetized silica** in the following proportion calculated per one sample: **10 µl** of **Internal Control (IC)**, **10 µl** of **Component A-1**, and **10 µl** of **Magnetized silica**. Prepare the mixture for total number of test and control samples plus extra samples. For example:

Number of samples for DNA extraction	Internal Control (IC), µl	Component A-1, µl	Magnetized silica, µl
6	70	70	70
12	130	130	130
18	190	190	190
24	250	250	250

NOTE: It is allowed to change the volume of **Internal Control (IC)** according to the Instruction manual for the used PCR kit.

3. Add **30 µl** of the thoroughly resuspended mixture of **Internal Control (IC)**, **Component A-1**, and **Magnetized silica** (or **20 µl** of the mixture in case of exogenous **IC** absence) into each tube with lysed cell pellet and the tubes for control samples. Recalculate the volume of mixture if the volume of Internal Control (IC) has been changed
4. Add **450 µl** of **Lysis Solution MS-URO** into the tubes with cell pellet and the tubes for control samples.
5. Add **100 µl** of **Negative Control (C–)** reagent into the tube for the Negative Control of extraction (C–) and **100 µl** of **required Positive Control sample** into the tube for the Positive Control of extraction (PCE) (if they are provided for PCR). Tightly close the tubes and thoroughly mix by vortexing.

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.

6. Incubate the tubes at **60 °C** for **10 min** in a thermostat.
7. Mix the contents of the tubes, then sediment the drops by short centrifugation. Transfer the tubes to a magnetic rack, and incubate for **2 min**.

NOTE: If it is inconvenient/impossible to open the tubes in a magnetic rack without sorbent resuspension, it is necessary to open the tubes before placing them in a magnetic rack.

8. Leaving the tubes in the magnetic rack, carefully remove the supernatant inserting the tip near the internal tube wall and using vacuum aspirator and a new tip for each sample.
9. Add **200 µl** of **Washing Solution 4** to the tubes. Tightly cap the tubes.

NOTE: If it is inconvenient/impossible to tightly close the tubes in a magnetic rack it is necessary to place the tubes in a regular tube rack.

10. Resuspend **Magnetized silica** by mixing on vortex. Then sediment the drops by short centrifugation.
11. Transfer the tubes to a magnetic rack for **1 min**.
12. Open the tubes, then carefully remove the supernatant according to the item 10.
13. Dry the magnetized silica. To do this, open the tubes and incubate them in a thermostat at **60°C** for **5 min**. In case of incomplete drying of the sorbent, it is allowed to increase the time up to **10 min**.
14. Add **100 µl** of **Buffer for elution B** into each tube, close the tubes and mix on vortex.

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

15. Transfer the tubes into a thermostat at **60 °C** for **5 min,** mix by vortexing after **2 min**.
16. Sediment the drops by short centrifugation and transfer the tubes to a magnetic rack. Incubate for **2 min**. Supernatant contains purified DNA.

NOTE: Do not take the tubes away from a magnetic rack when removing DNA.

Procedure in case of using automated station for nucleic acid extraction

The detailed information work with automated stations see in the *Instruction Manual* given by the manufacturer of the instrument.

Extraction using stations with magnetic stirring (for example, Auto-Pure 96 (Hangzhou Allsheng Instruments Co.,Ltd., China), KingFisher Flex (Thermo FS, Finland)).
Only the protocols located on the amplisens.ru website should be used for extraction.

NOTE: The extraction protocol for the automated station contains specific parameters that are not available for manual extraction.

1. If there is a precipitate in **Lysis Solution MS-URO** warm it up at 60 °C until crystals disappear. Resuspend **Magnetized silica** on vortex.
2. Prepare the required number of 96-well plates (see point 3) compatible with the instrument; mark them according to the protocol.
3. Add in the plates according to the marking and quantity of samples for extraction:
   - **450 µl** of **Lysis Solution MS-URO** per deep well plate,
   - **200 µl** of **Washing Solution 4** per deep well plate,
   - **100 µl** of **Buffer for elution B** per the plate for elution.

   NOTE: It is allowed to change the elution volume according to the Instruction manual for the used PCR kit.
4. Mix in a separate tube in the following proportion calculated per one sample:
   - **10 µl** of **Internal Control (IC)** (if it is provided for analysis),
   - **10 µl** of **Component A-1**,
   - **10 µl** of **Magnetized silica**.

Prepare mixture for total number of test and control samples plus extra samples (for example, in case of extraction from 96 samples it is recommended to prepare mixture for 100 extractions, i.e. plus 4 extra samples).

NOTE: It is allowed to change the volume of **Internal Control (IC)** according to the Instruction manual for the used PCR kit.

5. Add **30 µl** (or **20 µl** in case of exogenous Internal control absence) of thoroughly resuspended mixture to each well with **Lysis Solution MS-URO**. Recalculate the volume of mixture if the volume of Internal Control (IC) has been changed.
6. Add the whole content of the tubes with lysed cell pellet and **100 µl of control samples** (if they are provided for analysis) into each well with **Lysis Solution MS-URO** and mixture according to the marking.
7. Choose the protocol of extraction, place the prepared plates and tip-plate according to the protocol and placement indicators.
8. Run the DNA extraction protocol.
9. If necessary, seal the elution plate with the purified DNA using a self-adhesive film at the end of the extraction.

Extracted DNA can be stored:
- at 2-8 °C for 1 week,
- at the temperature from minus 24 to minus16 °C for 1 year.

In case of manual extraction transfer the supernatant for purified DNA storage into a new tube without disturbing the pellet.
In case of automatic extraction transfer eluate into the tubes for purified DNA storage at subzero temperature.

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 further questions or if encounter problems, please contact our Authorized representative in the European Community.

10. TRANSPORTATION

AmpliSens® MAGNO-sorb-URO nucleic acid extraction kit should be transported at 2-25 °C for no longer than 5 days.

11. STABILITY AND STORAGE

All components of **AmpliSens® MAGNO-sorb-URO** nucleic acid extraction kit are to be stored at 2-8 °C, when not in use (except for Internal Control-FL (IC)). All components of **AmpliSens® MAGNO-sorb-URO** 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. Karpov S.I., Matveeva M.V., Selemenev V.F. // Russian Journal of Physical Chemistry A. 2001. Vol. 75, No 2, pp. 266-271;
2. Sameer A., Barghouthi A // Indian J. Microbiol. 2011. Vol. 51, pp. 430-444;
3. Chiang C.-L., Sung C.-S., Wu T.-F., et al. Application of Superparamagnetic Nanoparticles in Purification of Plasmid DNA from Bacterial Cells // J. Chromatogr. B. 2005. V. 822. P. 54-60.

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® MAGNO-sorb-URO** 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
18.10.19 PM	Through the text	The text formatting was changed
	3.Content	The colour of the reagents was specified
26.05.20 VA	Footer	The phrase "Not for use in the Russian Federation" was added
11.03.21 VA	—	The name, address and contact information for Authorized representative in the European Community was changed
08.06.22 KK	1. Intended use	The intended use was specified. The list of biological material was expanded. The subsection of «Indications and contra-indications for use of the reagent kit» was added
	Through the text	The variant 100C was changed to variant 100. Lysis Solution MAGNO-sorb was changed to Lysis Solution MS-URO, Washing solution 7 was changed to Washing solution 4. The variant 100D was added
	3. Content	Volumes and quantity of Component A-1, Magnetized silica and Buffer for elution B were changed for variant 100
	4. Additional requirements	The automatic stations for nucleic acid extraction was added and additional materials if they are used were added
	5. General precautions	In accordance with Regulation 1272/2008/EC, hazard identification information for Lysis Solution MS-URO, Washing solution 4 and Mucolysin reagents were added
	6. Sampling and handling	"Interfering substances and limitations of using test material samples" and "Potential interfering substances" subsections were added
	7. Working conditions	The working conditions were changed from "18–25 °C" to "the temperature from 20 to 28 °C and relative humidity from 15 to 75 %"
	8. Protocol	The procedure of manual extraction using magnetic racks was clarified. The procedure of nucleic acid extraction using automated stations was added
	11. Stability and storage	The storage temperature range was changed from 2-25 ºC to 2-8 ºC. The information about storage temperature of Internal Control-FL (IC) was deleted
	12. References	The section 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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