CE

IVD

For Professional Use Only

MAGNO-sorb

Nucleic Acid Extraction Kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of
Science “Central Research
Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

TABLE OF CONTENTS

1. INTENDED USE

MAGNO-sorb nucleic acid extraction kit is intended for extraction of DNA/RNA from human blood plasma with subsequent detection of *hepatitis B virus, hepatitis C virus, human immunodeficiency virus*, and other pathogens by polymerase chain reaction (PCR).

2. PRINCIPLE AND PROCEDURE

Extraction and purification using MAGNO-sorb reagent kit is based on a simple bind-wash-elute procedure. Nucleic acids are isolated from lysates through binding to the magnetic particles in the presence of a chaotropic salt, which removes water from hydrated molecules in solution. The particles are separated from the lysates using a magnet. The nucleic acids are then efficiently washed and eluted in small volumes of elution buffer. The obtained nucleic acid sample is highly purified and free from inhibitors of amplification, which provides high analytical sensitivity of PCR assay.

3. CONTENT

MAGNO-sorb nucleic acid extraction kit is produced in 2 forms:

MAGNO-sorb nucleic acid extraction kit variant 100-200, **REF** K2-16-200-CE.

MAGNO-sorb nucleic acid extraction kit variant 100-1000, **REF** K2-16-1000-CE.

MAGNO-sorb nucleic acid extraction kit variant 100-200 or 100-1000 includes:

Reagent	***Description***	***Variant 100-200***		***Variant 100-1000***	
		Volume, ml	***Quantity***	***Volume, ml***	***Quantity***
Lysis Solution MAGNO-sorb	colorless clear liquid[1]	25	4 vials	70	4 vials
Component A	colorless clear liquid	0.3	4 tubes	0.6	4 tubes
Washing Solution 5	colorless clear liquid[1]	60	4 vials	60	4 vials
Washing Solution 6	colorless clear liquid	20	4 vials	20	4 vials
Washing Solution 7	colorless clear liquid	6.0	4 vials	6.0	4 vials
Magnetized silica	Black suspension	0.6	4 tubes	0.9	4 tubes
Buffer for elution	colorless clear liquid	1.2	12 tubes	1.2	12 tubes

MAGNO-sorb nucleic acid extraction kit variant 100-200 is intended for DNA/RNA extraction from 100 samples (including controls). The volume of test material is 200 µl.

[1]If Lysis Solution MAGNO-sorb and Washing Solution 5 are stored below 20 °C, crystalline precipitate may form.

MAGNO-sorb nucleic acid extraction kit variant 100-1000 is intended for DNA/RNA extraction from 100 samples (including controls). The volume of test material is 1,000 µl.

4. ADDITIONAL REQUIREMENTS

- Disposable powder-free gloves and laboratory coat;
- Pipettes (adjustable);
- Sterile DNase- and RNase-free pipette tips with aerosol barriers (from 200 to 1,000 µl),
- Sterile DNase- RNase-free pipette tips without aerosol barriers (up to 200, 1,000, and 5,000 µl),
- Tube and tip racks;
- Magnetic racks for 1.5- and 5-ml tubes;
- Vortex mixer;
- Desktop microcentrifuge;
- PCR box or biological cabinet;
- Vacuum aspirator with flask for removing supernatant;
- 1.5-ml disposable polypropylene tubes;
- 5-ml disposable polypropylene or polystyrene tubes, 12 mm diameter;
- Refrigerator for 2–8 °C;
- Thermostat for 1.5- and 5-ml tubes with controlled temperature and capable of incubating at 25-100 °C;
- Waste bin for used tips;
- Permanent pen for labeling.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile DNase- RNase-free pipette tips with aerosol barriers and use new tip for every procedure.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. 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 samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet

in accordance 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 contact with the skin, eyes and mucosa. If skin, eyes and mucosa 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/RNA extraction.
- 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.

 F Xi Xn	**Lysis Solution MAGNO-sorb**	Contains isopropyl alcohol. Flammable (F). Irritant (Xi). Contains guanidine thiocyonate. Harmful (Xn). Risk and safety phrases: R11-20/21/22-32-36-52/53-67 S7-13-16-24/25-26-36-46-61
 F Xn	**Washing Solution 5**	Contains ethanol. Flammable (F). Contains guanidine thiocyanate. Harmful (Xn) Risk and safety phrases: R11-20/21/22-32-52/53 S2-7-13-16-26-36-46-61
 F	**Washing Solution 6**	Contains ethanol. Flammable. Risk and safety phrases: R11 S2-7-16
 F Xi	**Washing Solution 7**	Contains acetone. Flammable (F). Irritant (Xi). R11-36-66-67 S2-9-16-25-26-37-51-60

Risk phrases:

R11 Highly flammable.
R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
R32 Contact with acids liberates very toxic gas.
R36 Irritating to eyes.
R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
R66 Repeated exposure may cause skin dryness or cracking.
R67 Vapours may cause drowsiness and dizziness.

Safety phrases:

S2 Keep out of the reach of children.
S7 Keep container tightly closed.
S9 Keep container in a well-ventilated place.
S13 Keep away from food, drink and animal feeding stuffs.
S16 Keep away from sources of ignition - No smoking.
S24/25 Avoid contact with skin and eyes.
S25 Avoid contact with eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36 Wear suitable protective clothing.
S37 Wear suitable gloves.
S46 If swallowed, seek medical advice immediately and show this container or label.
S51 Use only in well-ventilated areas.
S60 This material and its container must be disposed of as hazardous waste.
S61 Avoid release to the environment. Refer to special instructions safety data sheet.

6. SAMPLING AND HANDLING

Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

MAGNO-sorb nucleic acid extraction kit is recommended for **DNA** and **RNA** extraction from blood plasma.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1 Extraction from a blood plasma sample of 1,000 µl

1. Warm up **Lysis Solution MAGNO-sorb** and **Washing Solution 5** at 60 ºC until crystals disappear.
2. Prepare the required number of disposable 5-ml tubes (including a tube for the Negative Control of extraction, C–, and a tube for the Positive Control of Extraction, PCE, if provided with the amplification kit) and tube caps. Mark the tubes.
3. DNA/RNA extraction from 24 samples:
   a) add the required volume of the **Internal Control** sample, IC, (if provided with the amplification kit), the entire content of the tube with **Component A (0.6 ml)**, and the entire content of the tube with **Magnetized silica (0.9 ml)** to the vial with **Lysis Solution MAGNO-sorb (70 ml)**;
   b) cap the vial and gently turn it upside down 5-7 times to mix the content. Avoid foaming;
   c) transfer **2.6 ml** of the prepared mixture of **Lysis Solution MAGNO-sorb**, **IC**, **Component A**, and **Magnetized silica** to 5-ml tubes.
4. DNA/RNA extraction from less than 24 samples:
   a) mix in a disposable 1.5-ml tube **Internal Control** sample, IC, (if provided with the amplification kit), **Component A**, and **Magnetized silica** in the following proportion calculated per one sample: 10 µl of IC, 20 µl of Component A, and 30 µl of Magnetized Silica. Do not forget to add extra volumes for one more reaction. For example:

Number of samples to be extracted	IC, µl	Component A, µl	Magnetized silica, µl
6	70	140	210
12	130	260	390
18	190	380	570

b) transfer **60 µl** of the prepared mixture of IC, Component A, and Magnetized silica to the 5-ml tubes;

c) add **2.6 ml** of **Lysis Solution MAGNO-sorb** to the 5-ml tubes.

5. Transfer **1 ml** of a **plasma sample** to each tube containing the lysis solution**.** Mix by pipetting. Cap the tubes.
6. If it is required by the amplification kit, prepare the Positive Control of extraction, **PCE**. To do this, add **1 ml** of the provided **Positive Control sample** to the tube containing lysis solution. If the volume of the provided Positive Control sample is less than 1 ml, bring it to 1 ml by adding the Negative Control sample. Mix by pipetting. Cap the tube.
7. Prepare the Negative Control of extraction, **C–**, for each examined panel. To do this, add 1 ml of the Negative Control sample to the tube containing lysis solution. Mix by pipetting. Cap the tube.
8. Incubate the tubes at 60 °C for 10 min.
9. Transfer the tubes to a magnetic rack and incubate for **6 min**.
10. Carefully remove the supernatant inserting the tip near the internal tube wall and using vacuum aspirator. Take a new 1-ml tip for each sample. Transfer the tubes to a regular tube rack.
11. Add **700 µl of Washing Solution 5** to the tubes. Cap the tubes.
12. Take the required number of disposable 1.5-ml tubes (including the tubes for the Positive and Negative Controls of extraction). Mark the tubes.
13. Vortex the tubes and then pipette to remove magnetic beads from tube walls. Transfer the entire content of the tubes to the prepared 1.5-ml tubes.
14. Place the tubes to the magnetic rack and incubate for **2 min**.
15. Remove the supernatant and transfer the tubes to a regular tube rack.
16. Add **700 µl** of **Washing Solution 5**, vortex the tubes to resuspend magnetized silica, remove drops by short centrifuging, and then repeat steps 14-15.
17. Carry out washing procedure with **700 µl** of **Washing Solution 6** as described above.
18. Add **200 µl of Washing Solution 7**, mix, and vortex shortly to remove drops.
19. Place the tubes to the magnetic rack for **1 min** and then remove the supernatant.
20. Dry the sorbent. To do this, open the tubes and incubate them in the magnetic rack for **10 min**.
21. Add **50 µl** of **Buffer for elution** to each tube and vortex. Elution volume can be brought to up to 100 µl.
22. Incubate the tubes at 60 °C for 5 min. Vortex in 2 min.
23. Vortex the tubes shortly to remove drops and transfer the tubes to the magnetic rack.

Incubate for 2 min. Supernatant contains purified DNA and RNA.

Do not take the tubes away from a magnetic rack when removing DNA/RNA for RT-PCR

After transferring supernatant to the tubes, DNA samples can be stored:

- at 2–8 °C for 1 week;
- at ≤ –16 °C for 1 year;

After transferring supernatant to the tubes, RNA samples can be stored:

- at 2–8 °C for 4 hours;
- at ≤ –16 °C for 1 month;
- at ≤ –68 °C for 1 year.

8.2 Extraction from a blood plasma sample of 200 µl

1. Warm up **Lysis Solution MAGNO-sorb** and **Washing Solution 5** at 60 °C until crystals disappear.
2. Prepare the required number of disposable 1.5-ml tubes (including a tube for the Negative Control of extraction, C–, and a tube for the Positive Control of Extraction, PCE, if provided with the amplification kit). Mark the tubes.
3. Mix in a disposable 1.5-ml tube **Internal Control** sample, IC, (if provided with the amplification kit), **Component A**, and **Magnetized silica** in the following proportion calculated per one sample: 10 µl of IC, 10 µl of Component A, and 20 µl of Magnetized silica. Do not forget to add extra volumes for one more reaction. For example:

Number of samples to be extracted	IC, µl	Component A, µl	Magnetized silica, µl
6	**70**	**70**	**140**
12	**130**	**130**	**260**
18	**190**	**190**	**380**
24	**250**	**250**	**500**

4. Transfer **40 µl** of the prepared mixture of IC, Component A, and Magnetized silica to the tubes.
5. Add **900 µl** of **Lysis Solution MAGNO-sorb** to the tubes.
6. Transfer **200 µl** of a **plasma sample** to each tube containing the lysis solution**.** Mix by vortexing.
7. If it is required by the amplification kit, prepare the Positive Control of extraction, **PCE**. To do this, add **200 µl** of the provided **Positive Control** sample to the tube containing lysis solution. If the volume of the provided Positive Control sample is less than 200 µl, bring it to 200 µl, by adding the Negative Control sample. Mix by vortexing.
8. Prepare the Negative Control of extraction, **C–**, for each examined panel. To do this,

add 200 µl of the Negative Control sample to the tube containing lysis solution. Mix by vortexing.

9. Incubate the tubes at 60 °C for 10 min.
10. Centrifuge the tubes shortly, transfer the tubes to a magnetic rack, and incubate for **2 min**.
11. Carefully remove the supernatant inserting a tip near the internal tube wall and using vacuum aspirator. Take a new tip for each sample. Transfer the tubes to a regular tube rack.
12. Add **700 µl** of **Washing Solution 5** to the tubes.
13. Vortex the tubes to remove magnetic beads from tube walls then centrifuge shortly.
14. Place the tubes to a regular rack, open the tubes, and transfer to the magnetic rack. Incubate for **2 min**.
15. Remove the supernatant and transfer the tubes to a regular tube rack.
16. Repeat washing procedure with **Washing Solution 5** (steps 12-15).
17. Carry out washing procedure with **700 µl** of **Washing Solution 6** as described above.
18. Add **200 µl of Washing Solution 7**, mix, and vortex shortly to remove drops. Place the tubes to a regular rack and open the tubes.
19. Transfer the tubes to the magnetic rack for **1 min** and then remove the supernatant.
20. Dry the sorbent. To do this, open the tubes and incubate them in the magnetic rack for **10 min**.
21. Add **50 µl** of **Buffer for elution** to each tube and vortex. Elution volume can be brought to up to 100 µl.
22. Incubate the tubes at 60 °C for 5 min. Vortex the tubes in 2 min.
23. Vortex the tubes shortly and transfer them to the magnetic rack. Incubate for 2 min. Supernatant contains purified DNA and RNA.

Do not take the tubes away from a magnetic rack when removing DNA/RNA for RT-PCR

After transferring supernatant to the tubes, DNA samples can be stored:

- at 2–8 °C for 1 week;
- at ≤ –16 °C for 1 year;

After transferring supernatant to the tubes, RNA samples can be stored:

- at 2–8 °C for 4 hours;
- at ≤ –16 °C for 1 month;
- at ≤ –68 °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 zone 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 different Amplification areas.

10. TRANSPORTATION

MAGNO-sorb nucleic acid extraction kit should be transported at 2–8 °C for no longer than 5 days.

11. STABILITY AND STORAGE

All components of **MAGNO-sorb** nucleic acid extraction kit are to be stored at 2–25 °C, when not in use. All components of MAGNO-sorb nucleic acid extraction kit are to be stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

12. REFERENCES

1. 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 State Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Total Quality Management System, each lot of MAGNO-sorb 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	 Xn	Harmful
	Manufacturer	 Xi	Irritant
	Date of manufacture	 F	Flammable
PCE	Positive Control of extraction	**IC**	Internal Control
		C–	Negative Control of extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
22.10.12 LA	8.2 Extraction from a blood plasma sample of 200 µl	It was indicated that storage conditions for nucleic acid samples extracted from 200 µl of blood plasma are the same as for the samples extracted from 1,000 µl of blood plasma