ExtraMAG 2 DNA/RNA Isolation Kit

User manual

#EM3.2

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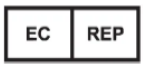

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Abbreviations

RT-PCR	Reverse transcription polymerase chain reaction
Real-time PCR-RT	Real-time polymerase chain reaction with fluorescence detection with reverse transcription
NA	Nucleic acid
PC	Positive control sample
NC	Negative control sample

1. INTENDED USE

ExtraMAG 2 DNA/RNA Isolation Kit is intended for the isolation of DNA or RNA from clinical material (oropharyngeal swab, nasopharyngeal swab, sputum) by automatic method based on the reversible binding of nucleic acid molecules on the surface of magnetic sorbent particles in the presence of chaotropic salts, for subsequent detection and identification of NA by polymerase chain reaction (PCR) or reverse transcription polymerase chain reaction (RT-PCR), in particular for diagnostics of SARS-CoV-2 infection.

Demographic and population aspects of the application. It is recommended to use ExtraMAG 2 for the preparation of clinical samples obtained from male and female patients without age restrictions.

The intended purpose of ExtraMAG 2 is to provide a preanalytical stage for the analysis of biological samples performed during the clinical laboratory diagnostics of infectious pathology using PCR or RT-PCR. The kit is used to obtain NA samples free of inhibitors of reverse transcription and PCR, thus ensuring high analytical capabilities for subsequent analysis.

The material for the NA extraction procedure is clinical material (oropharyngeal swab, nasopharyngeal swab, sputum).

Application. Professional use in clinical laboratory diagnostics and research practice. For *in vitro* diagnostics only.

Users' qualification requirements. A specialist with a higher or secondary specialized medical education, trained in licensed laboratory diagnostics courses.

2. PRINCIPLES OF THE PROCEDURE

The working principle of ExtraMAG 2 is based on the reversible binding of nucleic acid molecules on the surface of magnetic sorbent particles. The sample is treated with a lysis solution in the presence of magnetic sorbent particles. As a result, cell membranes, viral membranes and other biopolymer complexes are destroyed, and nucleic acids are released. Dissolved NAs link with the sorbent particles, while the other components of the lysed biological material remain in solution and are removed during magnetic precipitation of sorbent on a magnetic stand followed by washing of the sorbent. When an elution buffer is added to the magnetic sorbent, NAs are eluted from the surface of the sorbent into solution, which is then separated from the sorbent particles by magnetic force. As a result of this procedure, a highly purified NA sample free from amplification reaction inhibitors is obtained, which ensures high analytical sensitivity of the PCR or RT-PCR study.

3. KIT DESCRIPTION

ExtraMAG 2 is for automatic use (for example, automatic stations for the isolation of NA KingFisher Flex, Thermo Fisher Scientific; the system for the automatic isolation of nucleic acids from human biological samples Auto-Pure 96 from Hangzhou Allsheng Instruments Co., Ltd or similar).

Reagents that are provided in this kit type are sufficient for 96 reactions. ExtraMAG 2 is not sterile.

Table 1. ExtraMAG 2 description

Kit Component	***Description***	***Amount***
Plate with Lysis Buffer and Magnetic Sorbent	96-well plate, each well contains 0.4 ml of suspension (black or brown particles in the clear liquid); after the plate stirring within 5–10 seconds – opaque uniform suspension of black or brown color	1 piece
Wash Buffer Plate	96-well plate with 0.7 ml colorless, clear liquid in each well	1 piece
Elution Buffer Plate	96-well plate with 0.1 ml colorless, clear liquid in each well	1 piece
Comb	Polypropylene 96 deep-well format plate comb	1 piece
User manual	-	1 piece
Certificate of quality	-	1 piece

A polypropylene plate for inserting the comb is not included.

4. PERFORMANCE CHARACTERISTICS

ExtraMAG 2 provides isolation from the clinical material of NAs with a A260/280 purity ratio of at least 1.7.

To control the quality of biomaterial sampling and NA extraction, it is recommended to use the quality controls of biomaterial sampling, which are part of the reagent kits for PCR or RT-PCR as a separate component or use reagent kits for real-time PCR or PCR-RT, which include a fluorescent probe for the detection of endogenous human genomic NA in the sample.

The influence of potentially **interfering substances** on the operation of the reagent kit was tested in relation to potentially interfering substances that will occur during the procedure for collecting biological material:

1. Hemoglobin 10% v/v
2. Mucin 5% v/v
3. "Ibuprofen" 0.04 mg/ml
4. "Ambrobene" 0.003 mg/ml
5. "Bromhexine" 0.016 mg/ml
6. "Kaletra" 0.02 mg/ml
7. "Interferon" 0.2 U/ml
8. "Teraflu" 0.071 mg/ml

These potentially interfering substances encountered in the procedure for isolating NA from clinical material, evaluated at concentrations that would be expected to occur during normal use of the reagent kit, do not interfere with the assay result.

5. CLINICAL MATERIAL RECOMENDATION

Clinical samples, including oropharyngeal swab, nasopharyngeal swab or sputum are to be used as clinical material for the extraction of nucleic acids.

According to WHO guidelines «Diagnostic testing for SARS-CoV-2» the optimal specimen depends on clinical presentation and time since symptom onset. Respiratory specimens:

• Upper respiratory specimens are adequate for testing early-stage infections, especially in asymptomatic or mild cases. Testing combined nasopharyngeal and oropharyngeal swabs from one individual has been shown to increase sensitivity for detection of respiratory viruses and improve the reliability of the result. Two individual swabs can be combined in one collection tube or a combined nasopharyngeal and oropharyngeal swab can be taken. A few studies have found that individual nasopharyngeal swabs yield a more reliable result than oropharyngeal swabs.

• Lower respiratory specimens are advised if collected later in the course of the COVID-19 disease or in patients with a negative URT sampling and there is a strong clinical suspicion of COVID-19. LRT specimens can consist of sputum, if spontaneously produced (induced sputum is not recommended as this poses an increased risk of aerosol transmission). Before implementing other respiratory or oral fluid sampling methods, the sampling method should first pass validation in the laboratory for the intended patient groups.

Specimens for virus detection should reach the laboratory as soon as possible after collection. Correct handling of specimens during transportation and in the laboratory is essential.

Specimen type	Collection materials	Recommended temperature for storage and/or shipment to the laboratory and until testing (from date of specimen collection) [#]
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs with VTM *	2-8 °C if ≤12 days* –70 °C (dry ice) if > 12 days
Sputum	Sterile container	2-8 °C if ≤ 2 days –70 °C (dry ice) if > 2 days

[#] Avoid repeated freezing and thawing of specimens. If no access to –70 °C consider storing at -20 °C.

*- * For transport of specimens for viral detection, use preferentially viral transport medium (VTM) containing antifungal and antibiotic supplements. If VTM is not available, other solutions may*

be used after validation. Such solution may include phosphate buffered saline (PBS), 0.9% sterile saline, minimum essential medium (with storage at +4C up to 7 to 14 days). In case other viruses such as influenza should also be tested, do not store samples for more than 5 days at 4-8 degrees but –70 °C or dry ice.

6. MATERIALS REQUIRED BUT NOT SUPPLIED*

- Laminar box II or III class of biological safety (for example, "BAVp-01-"Laminar-S"-1,2", "Laminar systems", Russia);
- Mini-Centrifuge/Vortex (for example, FV-2400 Micro-Spin, SIA Biosan, Latvia);
- Bench–top centrifuge for of the Eppendorf type microtubes (1.5–2 ml) up to 10000 g (for example, MiniSpin®, Eppendorf, Germany);
- Magnetic particle processor with a processing volume of 20–1000 µl (96-well plate with deep wells) or 200–5000 µl (24-well plate with deep wells), with a capacity of up to 96 samples (96-well plate with deep wells) or 24 samples (24-well plate with deep wells), particle extraction efficiency > 95%, for example, KingFisher Flex, KingFisher Flex, Finland);
- System for automatic isolation and purification of nucleic acids from human biological samples Auto-Pure 96 (Hangzhou Allsheng Instruments Co., Ltd, China);
- Polypropylene plate for inserting the comb;
- Refrigerator with chambers that maintain a temperature of +2 to +8 °C (for storing the "ExtraMAG 2" reagent kit);
- Refrigerator with a chamber that maintains a temperature of +2 to +8 °C (for storing the NA samples). Storage of the NA samples in the same refrigerator with the components of the NA isolation kit is not allowed;
- Eppendorf type microtubes (1.5–2 ml), with Safe-Lock (for example, Axygen, USA);
- Microtube rack (for 1.5–2 ml) (for example, Axygen, USA);
- 1-channel mechanical pipettes with a variable dosing volume of 2–20 µl, 5–50 µl, 20–200 µl, 100–1000 µl certified by the average dose value and the repeatability of pipetting results (error no more than 3%) (for example, Sartorius Biohit, Finland);
- Disposable tips with a filter for semi-automatic pipettes marked "RNAase-free, DNAase-free" in volumes of 1–20 µl, 5–50 µl, 20–200 µl, 100–1000 µl (for example, Axygen, USA);
- Personal protective equipment (PPE) (disposable dressing gown, hat, mask, gloves);
- Container with a lid for a disinfectant solution

** In the absence of the above equipment and materials, it is allowed to use other means with characteristics no worse than those given.*

7. INSTRUCTIONS FOR USE

7.1. Preparation of the components of ExtraMAG 2 for analysis.

Table 2. Preparation of the components for analysis

Kit component	*Preparation of the components*
Plate with Lysis Buffer and Magnetic Sorbent	Ready to use
Wash Buffer Plate	Ready to use
Elution Buffer Plate	Ready to use
Comb	Ready to use

7.2. Isolation of NA

ATTENTION! Before work, it is necessary to read the operating instructions for the KingFisher Flex automatic station.

7.2.1. Program the device in accordance with the operating instructions for the KingFisher Flex automatic station (script is provided by the manufacturer upon request).

7.2.2. Remove the protective film from the Plate with Lysis Buffer and Magnetic Sorbent.

7.2.3. Dispense 100 µl of clinical samples into the wells, 100 µl of PC* into a separate well for the

positive control, and 100 µl of NC* into a separate well for the negative control, according to the procedure.

** - included in kits for PCR or RT-PCR.*

7.2.4. Remove the protective foil from the Wash Buffer Plate and from the Elution Buffer Plate and prepare an empty plate (not supplied) with an attached comb for loading.

7.2.5. Switch on the device, select the User protocols tab in the main menu, then DNA/RNA, select the EM3-96-KF program (the file is provided by the manufacturer upon request). In accordance with the instructions of the EM3-96-KF program, load the plates into the device for automatic isolation, where:

- tip-plate – an empty plate (not supplied) with an attached comb;
- elution – plate with elution buffer;
- wash1 – plate with wash buffer;
- sample – plate with lysis buffer and magnetic sorbent and added samples

After the end of the extraction program, remove the plates from the device.

7.2.6. The elution plate contains the highly purified NA preparation. Lysis Buffer Plate, Wash Buffer Plate and the empty comb plate has to be disposed.

NA samples can be stored for 30 min at temperatures from + 2 °C to +8° C or for 1 week at a temperature not higher than minus 16 °C.

8. POSSIBLE PROBLEMS AND THEIR SOLUTION

8.1. Absence of a positive reaction with a known positive sample during PCR or RT-PCR.

№	Possible reason	Solution
1	Incomplete cell lysis due to the presence of a crystalline precipitate in the lysis buffer	Before each isolation procedure, it is necessary to stir the Lysis Buffer thoroughly. If crystals appear, warm up the vial at +65 °C until they are completely dissolved
2	Incorrect sample preparation due to non-compliance with recommendations for the procedure for obtaining clinical samples (including violation of storage and transportation conditions)	Take new samples and repeat the analysis
3	Insufficient amount of biological material in the sample	Take new samples and repeat the analysis

8.2. The presence of a positive reaction with a known negative sample during PCR or RT-PCR.

№	Possible reason	Solution
1	Contamination at the stage of NA extraction	Decontaminate, use filter tips, chemical and ultraviolet disinfection of all work surfaces, use separate sets of dispensers, equipment, gowns and gloves for each area, conduct internal and external quality control of studies

9. REAGENT HANDLING AND STORAGE

9.1. Storage conditions

Store ExtraMAG 2 at temperatures from + 2 to +8 °C. Do not use the kits stored in violation of the regulated regime.

9.2. Transportation conditions

ExtraMAG 2 can be transported by covered transport (road, rail or air) in thermal containers with refrigeration elements at temperatures from +2 to +8 °C. Do not use the kits transported in violation of the temperature regime.

9.3. Product shelf life

ExtraMAG 2 shelf life is 12 months.

9.4. Storage conditions and shelf life of opened product components.

№	Component	Storage conditions and shelf life
1	Plate with Lysis Buffer and Magnetic Sorbent	Not stored
2	Wash Buffer Plate	Not stored
3	Elution Buffer Plate	Not stored

10. WARNINGS AND SPECIAL PRECAUTIONS REGARDING THE SAFE DISPOSAL OF REAGENT KIT

10.1. Only personnel trained in molecular diagnostics is allowed to work with the kit of reagent. The rules for working in a clinical diagnostic laboratory have to be observed.

10.2. To prevent contamination of newly investigated samples, reagents and consumables with amplification products (amplicons), nucleic acid preparations or biomaterials, and, as a consequence, the appearance of false positive results, the laboratory process should be unidirectional. Separate rooms (zones) are used for different stages of the analysis. Work should start in the isolation zone, continue in the amplification and detection zones. Do not return samples, equipment and reagents to the area where the previous step of the process was carried out.

10.3. ExtraMAG 2 is intended for single use when the specified number of samples is to be isolated.

10.4. It is allowed to use the kit for the intended purpose only, in accordance with these instructions and within the indicated expiration date.

10.5. Do not use the reagent kit if the inner packaging is broken or the appearance of the reagent does not correspond to the description.

10.6. Do not use the reagent kit if the conditions of transportation and storage have not been observed according to the instructions.

10.7. In the process of work, it is imperative to use personal protective equipment: disposable gloves, laboratory coats. Wash hands thoroughly after finishing work.

10.8. When working with the kit, avoid contact of the kit components with the skin, eyes and mucous membranes. In case of contact, immediately rinse the affected area with water and seek medical attention.

10.9. The component of the kit "Wash Buffer Plate" contain isopropyl alcohol and acetone, which are classified as flammable liquids. Electrical equipment and lighting when working with isopropyl alcohol and acetone must be explosion-proof.

10.10. The component of the kit "Plate with Lysis Buffer and Magnetic Sorbent" contain guanidine thiocyanate, which can be absorbed through the skin and is a sensitizing agent. In case of contact with skin or eyes, immediately rinse these areas of the body with water.

10.11. Disposal of the reagent kit. Unused reagents, expired reagents, as well as used reagents, packaging, biological material, including materials, tools and items contaminated with biological material, should be disposed in accordance with the requirements of Directive 2008/98/EC.

The choice of the method of disinfection / neutralization is determined by the capabilities of the organization carrying out medical activities and is carried out when developing a scheme for handling medical waste. In the absence of a disinfection / neutralization site or a centralized medical waste disposal system adopted in the administrative territory in an organization carrying out medical activities; the kits are disinfected by the personnel of this organization in the places of their formation by chemical / physical methods.

The manufacturer, suppliers, sellers, importers can destroy kits that have lost their consumer properties or have expired, in accordance with Directive 2008/98/EC as class H9 medical waste using methods agreed with the territorial authorities responsible for sanitary and epidemiological well-being of the population. The personnel carrying out the disposal of the kits have to comply with the safety rules for carrying out one or another method of destruction.

11. LIMITATIONS

Contamination at the stage of NA isolation is a possible reason for obtaining a false positive result during the subsequent procedure of detecting and identifying NA by polymerase chain reaction (PCR) or reverse transcription polymerase chain reaction (RT-PCR).

12. MANUFACTURER'S WARRANTIES

12.1. ExtraMAG 2 is manufactured by LabPack LLC. Address: Karpovka River Embarkment, 5, letter I, room 3-N, 197376 Saint-Petersburg, Russia.
Phone: +7 (812) 490 75 93, e-mail: info@labpack.ru.

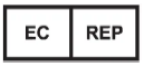
12.2. The manufacturer guarantees the compliance "ExtraMAG 2" reagent kit with the requirements of the technical documentation if the conditions of transportation, storage and use established by this instruction for use are observed.

12.3. Instruction manual is provided by the manufacturer on paper and in the form of an electronic document via online publishing. The latest revision of the instruction manual: 31/08/2021.

12.4. Complaints about the quality of ExtraMAG 2 are to be sent to an authorized representative of the manufacturer in the European Community:

MagnaLab LLC, Address: Ludwika Rydygiera Str. 8/4, Warsaw 01-793, Poland.
Phone: +48 531 261 817, e-mail: info@magnalab.pl

Appendix A. Symbols to be used with medical device labels

Symbol	Title of symbol	Symbol	Title of symbol
EN ISO 15223-1:2016			
	Use-by date		Do not re-use
	Date of manufacture		Consult instructions for use
	Batch code		Caution
	Catalogue number		*In vitro* diagnostic medical device
	Keep away from sunlight		Do not use if package is damaged
	Keep dry		Contains sufficient for <n> tests
	Temperature limit		Manufacturer
	Authorized representative in the European Community		
Regulation (EC) No 1272/2008			
	H225: Highly flammable liquid and vapour		
	H315: Causes skin irritation		

Appendix B. Harmonised standarts

1	EN ISO 13485:2016	Medical devices – Quality management systems – Requirements for regulatory purposes
2	EN 13612:2002	Performance evaluation of in vitro diagnostic medical devices
3	EN 13641:2002	Elimination or reduction of risk of infection related to in vitro diagnostic reagents
4	EN 13975:2003	Sampling procedures used for acceptance testing of in vitro diagnostic medical devices – Statistical aspects
5	EN ISO 14971:2012	Medical devices – Application of risk management to medical devices
6	EN ISO 15223-1:2016	Medical devices – Symbols to be used with medical device labels, labelling and information to be supplied – Part 1: General requirements
7	EN ISO 18113-1:2011	In vitro diagnostic medical devices – Information supplied by the manufacturer (labelling) – Part 1: Terms, definitions and general requirements
8	EN ISO 18113-2:2011	In vitro diagnostic medical devices – Information supplied by the manufacturer (labelling) – Part 2: In vitro diagnostic reagents for professional use
9	EN ISO 23640:2015	In vitro diagnostic medical devices – Evaluation of stability of in vitro diagnostic reagents
10	EN 62366-1-2015	Medical devices – Part 1: Application of usability engineering to medical devices
11	Directive 98/79/EC	Directive 98/79/EC of the European Parliament and of the Council on in vitro diagnostic medical devices
12	Directive 2008/98/EC	Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives

The above standards were valid at the time the instructions for use were approved. In the future, when using the document, it is advisable to check the validity of the reference normative documents at the current moment. If the referenced document is replaced or changed, then when applying this document, the replaced (modified) document should be used.