"MB-Si Kit2" reagent kit for the isolation of DNA/RNA from clinical material

Instruction for Users

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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

The "MB-Si Kit2" reagent kit is intended for the isolation of DNA or RNA from clinical material (oropharyngeal swab, nasopharyngeal swab, sputum) by an manual 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 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 the "MB-Si Kit2" reagent kit for the preparation of clinical samples obtained from male and female patients without age restrictions.

The intended purpose of the "MB-Si Kit2" reagent kit is to provide a preanalytical stage for the analysis of a biological sample performed during the clinical laboratory diagnostics of human infectious pathology using RT-PCR. The kit is used to obtain NA samples free of reverse transcription inhibitors and PCR, which provides 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 courses of specialization in clinical laboratory diagnostics.

2 PRINCIPLES OF THE PROCEDURE

The principle of the "MB-Si Kit2" reagent kit 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 NA links 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 sorbent. When an elution buffer is added to a magnetic sorbent, NA is 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 is obtained, free from amplification reaction inhibitors, which ensures high analytical sensitivity of the RT-PCR study.

3 KIT DESCRIPTION

The "MB-Si Kit2" reagent kit is for manual use. Reagents that are provided in this kit type are sufficient for 100 reactions. "MB-Si Kit2" reagent kit is not sterile.

Table 1. "MB-Si Kit2" reagent kit description

#EM3.2 "MB-Si Kit2" reagent kit (for automatic use)		
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 in "MB-Si Kit2" reagent kit.

- The "MB-Si Kit2" reagent kit 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.
- MB-Si Kit2" reagent kit is not sterile.

4 PERFORMANCE CHARACTERISTIC

"MB-Si Kit2" reagent kit provides isolation of NA with a purity with A260/280 ratio of at least 1.7 from the clinical material.

To control the quality of biomaterial sampling and NA extraction, it is recommended to use the quality control of biomaterial sampling, which are part of the reagent kits for RT-PCR as a separate component, or use reagent kits for real-time 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

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

The material for the extraction of nucleic acids is clinical samples, including oropharyngeal swab, nasopharyngeal swab or sputum.

ATTENTION! The container with the biomaterial is delivered to the laboratory and stored until the start of the study at +2 ... +8 °C. The time from taking the material to the start of the study should not exceed 24 hours. If a longer storage is required, place the material in a freezer at a temperature from minus 24 °C to minus 16 °C for up to 1 week; at a temperature not higher than minus 68 °C – for a long time. Only one freezing-thawing of the material is allowed.

Oropharyngeal swabs.

Sampling. Smears are taken with dry sterile cotton plastic-based swabs by rotational movements from the surface of the tonsils, palatine arches and posterior wall of the oropharynx. After taking the material, place the tampon (the working part of the probe with a cotton swab) in a sterile disposable tube with a special transport medium and the plastic rod is gently broken off at a distance of no more than 0.5 cm from the working part, leaving the working part of the probe with the material in the transport medium. Close the tube lid tightly.

Sample pretreatment. Not required.

Storage conditions for the material:

- at room temperature – for 6 hours;
- at a temperature of +2 to +8 °C – for 3 days;
- at a temperature of minus 20 °C – for 1 month;
- at a temperature of minus 70 °C – for a long time.

Only one freeze-thaw cycle is allowed.

Nasopharyngeal swabs.

Sampling. Smears (mucus) are taken with dry sterile cotton plastic-based swabs. The tampon is inserted with a light movement along the outer wall of the nose to a depth of 2–3 cm to the inferior concha. Then the tampon is slightly lowered downward, inserted into the lower nasal passage under the lower nasal concha, rotational movement is made and removed along the outer wall of the nose. After taking the material, place the swab (the working part of the probe with a cotton swab) in a sterile disposable tube with a snap-on lid containing the appropriate transport medium and gently break off the plastic rod at a distance of no more than 0.5 cm from the working part, leaving the working part of the probe with the material in the transport environment. Close the tube lid tightly.

Sample pretreatment. Not required.

Storage and transportation conditions:

- at room temperature – within 6 hours;
- at temperatures from +2 to +8 °C – for 3 days;
- at a temperature of minus 20 °C – for 1 month;
- at a temperature of minus 70 °C – for a long time.

Only one freeze-thaw cycle is allowed.

Sputum.

Sampling. The collection of material is carried out in an amount of at least 1.0 ml in disposable scaled sterile vials with a wide neck and screw caps with a volume of at least 50 ml.

Sample pretreatment. Before isolation of nucleic acids, it is necessary to dilute sputum using a Mucolysin solution (Na_2HPO_4 – 77.4 mM, NaH_2PO_4 – 22.6 mM, beta-ME – 99.4 mM, 5% sodium azide at a final concentration of 0.05%). Add "Mukolyzin" to the container with sputum with 5:1 ratio (5 parts of "Mukolyzin" to 1 part of sputum), comparing with scale on the container and sterile glass beads. In the process of liquefaction of sputum (20–30 minutes), the container is periodically shaken. Then, take 1 ml of liquefied sputum with an automatic pipette using a filter tip, placed in a tube with a screw cap or in a microcentrifuge tube with a 1.5 ml latch, and centrifuged at 5000–7000 g for 10 min. Remove 0.8 ml of the supernatant, mix the cell pellet with 0.2 ml of the remaining liquid. DNA can be isolated from 0.1 ml of diluted sputum without a centrifugation step.

Storage and transportation conditions for clinical material and pretreated samples:

- at room temperature – for 6 hours;
- at a temperature of +2 to +8 °C – for 3 days;
- at a temperature of minus 20 °C – for 1 week;
- at a temperature of minus 70 °C – for a long time.

6 MATERIALS REQUIRED BUT NOT SUPPLIED*

- Laminar box II or III class of biological safety
- Mini-Centrifuge/Vortex
- Bench–top centrifuge for of the Eppendorf type microtubes (1.5–2 ml) up to 10000 g
- 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 «MB-Si Kit2» 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
- Microtube rack (for 1.5–2 ml)
- 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%)
- 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
- 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 the "MB-Si Kit2" reagent kit for analysis.*

Table 2. Preparing components for analysis

#EM3.2 "MB-Si Kit2" reagent kit	
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 DNA*

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.

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 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 introduced 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 must 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 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 thoroughly mix the Lysis Buffer; if crystals appear, warm 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).	Repeat the selection on newly selected samples.
3	Insufficient amount of biological material in the sample.	Repeat the selection on newly selected samples.

8.2 *The presence of a positive reaction with a known negative sample during 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 "MB-Si Kit2" reagent kit at temperatures from + 2 to +8 °C. Kits stored in violation of the regulated regime are not subject to use.

9.2 *Transportation conditions*

"MB-Si Kit2" reagent kit can be transported by covered transport (road, rail or air) in thermal containers containing refrigeration elements at temperatures from +2 to +8 °C. Kits transported in violation of the temperature regime cannot be used.

9.3 *Product shelf life*

"MB-Si Kit2" reagent kit 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 ***To work with a set of reagent methods, only personnel trained in molecular diagnostics and the rules for working in a clinical diagnostic laboratory in the prescribed manner.***

10.2 ***In order to prevent contamination with amplification products (amplicons), nucleic acid preparations or biomaterials of newly investigated samples, reagents and consumables, 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 zone. Do not return samples, equipment and reagents to the area where the previous step of the process was carried out.***

10.3 ***"MB-Si Kit2" reagent kit is intended for single use when the specified number of samples is isolated.***

10.4 ***It is allowed to use the kit only strictly for the intended purpose, 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 ***Kit's components "Wash Buffer Plate" contain isopropyl alcohol and acetone, which is classified as flammable liquids. Electrical equipment and lighting when working with isopropyl alcohol and acetone must be explosion-proof.***

10.10 ***Kit's component "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 removed in accordance with the requirements of Directive 2008/98/EC.***

Wastes containing (used) microorganisms (materials, tools and items contaminated with biological fluids) must be decontaminated / neutralized.

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 destruction of the kits must 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 reverse transcription polymerase chain reaction (RT-PCR).

12. MANUFACTURER'S WARRANTIES

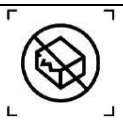
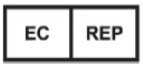
12.1 ***"MB-Si Kit2" reagent kit is manufactured by LabPack LLC. Address: Karpovka River Embarkment, 5, letter I, room 3-N, 197376 Saint-Petersburg, Russia. Phone: +7 (812) 490 75 93.***

12.2 ***The manufacturer guarantees the compliance "MB-Si Kit2" 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: 24/05/2021.***

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.