SaMag Extraction kit User Manual

for use with **SaMag-12** and **SaMag-24** automated extraction systems from Sacace Biotechnologies

- **SaMag Bacterial DNA Extraction Kit (SM006)**

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IVD

REF SM006

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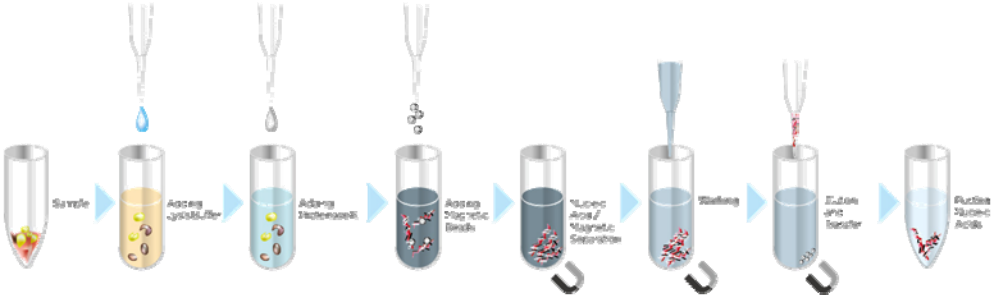
SaMag Bacterial DNA Extraction Kit

NAME
SaMag Bacterial DNA Extraction Kit

INTENDED USE
SaMag Bacterial DNA Extraction Kit is designed to be used with SaMag-12/24 automatic nucleic acid extraction system for extraction of genomic DNA from both Gram-positive and Gram-negative bacteria.

PRINCIPLE OF ASSAY
The extraction process consists of steps of lysis, binding, washing and elution as figure below.



The prepared nucleic acids are suitable for applications like qPCR, sequencing (NGS), Microarray, RFLP, Southern Blot or any kind of enzymatic manipulation.

MATERIALS PROVIDED
- Reagent cartridge, 48 pcs (6x8);
- Reaction chamber, 48 pcs (2x 6x4);
- Tip holder, 48 pcs (2x 6x4);
- Filtered tip, 50 pcs (50x1);
- Piercing pin, 50 pcs (50x1);
- Sample tube (2 ml), 50 pcs (50x1);
- Elute tube (1,5 ml), 50 pcs (50x1);
- Buffer BL2, 1 pc x 25ml;
- Barcode paper, 1 sheet;

Contains reagents for 48 tests.

MATERIALS REQUIRED BUT NOT PROVIDED
- SaMag-12/24 Automatic Nucleic Acids Extraction System (Sacace Biotechnologies, Italy)
- Disposable gloves, powderless
- Micropipettes
- Biological cabinet

PRODUCT USE LIMITATIONS
All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification. Strict compliance with the user manual is required for optimal results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

REAGENT CARTRIDGE CONTENT

well-1	Proteinase K solution	40 µl
well-2	Lysis Buffer 2	720 µl
well-3	Binding Buffer 1	720 µl
well-4	Magnetic Bead Solution	800 µl
well-5	Washing Buffer 1	1000 µl
well-6	Washing Buffer 2	1000 µl
well-7	Washing Buffer 3	1000 µl
well-8	Elution Buffer 1	1000 µl
well-9	Elution Buffer 2	1000 µl
well-10	Empty	

STORAGE

SaMag Bacterial DNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The kits are stable under such conditions up to expiration date.
Store the purified DNA at 4 °C (short- term) or aliquot and store at -70°C (long-term) before perform the downstream analysis.

WARNINGS AND PRECAUTIONS

- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with Biosafety Level 2 or other appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

STARTING MATERIAL

Bacterial pellet/colony from culture, cell-free body fluids, liquid transport media, urine, environment material (water, soil, etc.). To use the paraffin- embedded tissue sections as samples, we recommend to extract DNA with SaMag FFPE DNA Extraction kit (SM009).
To use tissue as samples, we recommended using the SaMag Tissue DNA Extraction kit (SM004).

SAMPLE PREPARATION

Sample preparation requirements are highly dependent upon the type of starting material. Due to variations in consistency and viscosity, even similar sample types may require distinct handling
The **buffer BL2 is specialized for bacterial cell wall lyse*** (Supplied in the kit), use it to resuspend the bacterial pellet before process extraction
* For *mycobacterium spp.*(e.g. MTB), use SaMag TB DNA Extraction kit (SM008).

Please follow the recommendations in processing the primary samples before nucleic acid extraction as described below:

For viscous samples (e.g. BAL, sputum or other mucous specimen)**:**

Recommended pretreatment : Liquefaction

- Prepare a fresh DTT stock solution for liquefaction * (e.g., 5× conc. DTT stock is about 0.75%);
- Adjust the final DTT concentration in the sample to 0.15% by adding DTT stock solution;
- Incubate the sample (e.g., with shaking at 850 r.p.m. for 30 min at 37°C) until it can be pipetted easily;
- Pellet bacteria by centrifugation at 14000 x g for 10 min;
- Discard supernatant, resuspend the pellet in 220 µl Buffer BL2;
- Transfer 200 µl suspension to sample tube (Supplied in the kit).

** The liquefaction could be done by using other solutions, such as NALC (N-Acetyl-L-Cysteine) -NaOH or other agents which could digest mucous material.*

For large volume liquid samples that have low or unknown bacterial loads (e.g. urine**, water collected from pool/river stream/tower)**:**

Recommended pretreatment : Centrifugation

- Centrifuge the sample for up to 10 min at 20,000 × g to concentrate the bacterial cells in pellet;
- Discard supernatant, resuspend the pellet in 220 µl Buffer BL2*;
- Take 200 µl supension to sample tube (Supplied in the kit).

** If there were sand or other visible particle in the pellet, centrifuge again after BL2 buffer treatment or filter out the dust is recommended*

*** for detection of sexually transmitted diseases (e.g. Chlamydia trachomatis) from urine we recommend to use SaMag STD DNA Extraction kit (code SM007)*

For cell-free body fluids (e.g. CSF, BAL, aspirates)**:**

Recommended pretreatment : Centrifugation

Method 1

- Pellet bacteria by centrifugation at 14000 x g for 10 min
- Resuspend bacterial pellet in 220 µl Buffer BL2
- Take 200 µl suspension to sample tube (Supplied in the kit)

Method 2 - Centrifugation free

- Take 200 µl sample in a 1.5 ml centrifuge tube
- Add 200 µl buffer BL2 to sample (1:1)
- Vortex-mixing for 5-10sec
- Transfer 400 µl sample to sample tube (Supplied in the kit)

For swab samples (e.g. eye, nasal, pharyngeal, or other swabs*)**:**

Method 1

- Collect samples and place in 2 ml PBS sterile. Incubate for 30min at room temperature;
- Pellet bacteria by centrifugation at 14000 x g for 10 min;
- Resuspend bacterial pellet in 220 µl Buffer BL2 (Supplied in the kit);
- Take 200 µl suspension to sample tube (Supplied in the kit).

Method 2- centrifuge free

- Place the sample swab in 440 µl buffer BL2, incubate for 30min at room temperature. Transfer 400 µl to sample tube.

**for urogenital swabs we recommend to use the kit SaMag STD DNA Extraction kit (code SM007)*

Enhance lysis efficiency of certain bacteria (e.g. Gram-positive species)**:**

Recommended pretreatment : Enzymatic Digestion

- Pellet bacteria by centrifugation at 14000 x g for 10 min;
- Suspend bacterial pellet in 200 µl of the appropriate enzyme solution*;
- Incubate for at least 30 min at 37°C;
- Add 220 µl Buffer BL2. Mix by vortexing;
- Take 200 µl suspension to sample tube (Supplied in the kit).

** enzyme solution: 20 mg/ml lysozyme or 200 µg/ml lysostaphin; 20 mM Tris-HCl, PH 8.0; 2 mM EDTA; 1.2% Triton)*

For some gram-positive bacterial species. Especially for samples that contain particles (e.g. stool)**:**

Recommended pretreatment : Mechanical homogenization

- Follow the regular homogenization procedures in the laboratory.
- For some sample types, DNA yield can be improved by performing this homogenization step prior to add buffer BL2 and proteinase K

Isolation of genomic DNA from bacterial suspension cultures:

- Pipet 1 ml of bacterial culture into a 1.5 ml microcentrifuge tube and centrifuge at 5000xg for 5 min;
- Discard supernatant;
- Add 220 µl Buffer BL2 to pellet and mix by vortexing for 5-10 sec;
- Take 200 µl suspension to sample tube (Supplied in the kit).

Isolation of genomic DNA from bacterial plate culture:

- Take 1-3 bacterial colony from culture plate with an inoculation loop and suspend in 220 µl of buffer BL2 by vigorous stirring;
- Take 200 µl suspension to sample tube (Supplied in the kit).

To inactivate pathogenic organisms in the sample:

Recommended pretreatment : Boiling

- Incubate samples at 95°C for 10 min
- Centrifuge briefly to collect the complete sample volume at the bottom of the tube.
- Allow samples to cool down or chill on ice, then transfer 100-400 µl of cooled sample to the sample tube.

PROTOCOL

To perform extraction start SaMag-12/24 instrument, open door(s) and follow steps indicated in SaMag user manual in chapter “Extraction”.

1. Insert cartridge(s)
2. **Insert Reaction Chamber(s) ***
3. Insert tip holder(s)
4. Insert piercing pin(s)
5. Insert filtered tip(s)
6. Insert Sample Tube(s) in sample rack
7. Insert 1,5 ml Elute tube(s) in sample rack, with open cap
8. Under a safe biological cabinet load Sample(s) in Sample tube(s)
9. If provided with the amplification kit, add Internal Control
10. Transfer sample rack into SaMag instrument
11. Close SaMag-12/24 door(s)
12. Use the barcode to select Bacterial DNA Extraction kit Protocol, appropriate Starting Volume, Elution Volume (suggested values are 400 µl for sample volume, 50 µl for elution volume).

12 bis. In case of using SaMag-12 ver. 3.x EVO please use the touchscreen interface to select the Bacterial DNA Extraction kit (code 2006).

NOTE: In case of using SaMag-12 ver. 3.x EVO please select the 2 ml rack type in the touchscreen interface.

DNA extracted with SaMag Bacterial DNA Extraction Kit is stable for up to one year when stored at –20°C, store it at –70°C or below for longer periods.

*** ALWAYS REMEMBER TO INSERT REACTION CHAMBERS FOR ALL LOADED SAMPLES, OTHERWISE BUFFERS MAY SPILL OUT DAMAGING THE INSTRUMENT, AND IN THAT CASE SACACE BIOTECHNOLOGIES WILL NOT BE HELD RESPONSIBLE.**

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