SaMag Extraction kit User Manual

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

- **SaMag TB DNA Extraction Kit (SM008)**

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IVD

REF SM008

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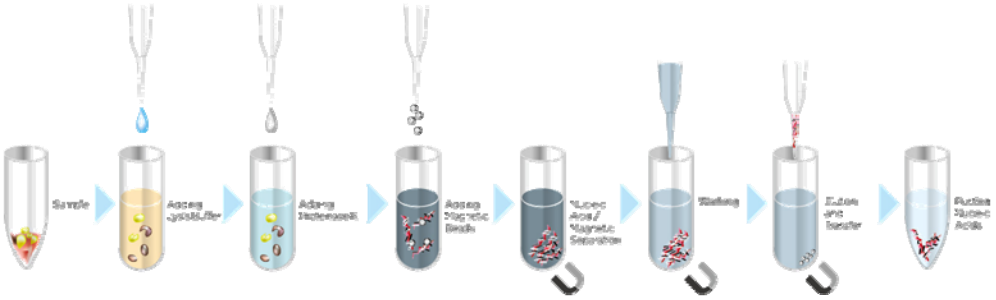
SaMag TB DNA Extraction Kit

NAME
SaMag TB DNA Extraction Kit

INTENDED USE
SaMag TB DNA Extraction Kit is designed to be used with SaMag-12/24 automatic nucleic acid extraction system for extraction of genomic DNA of *Mycobacteria* spp. (e.g. *Mycobacterium tuberculosis*) from different specimen.

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 BL3, 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	Buffer N1	300 µl

STORAGE

SaMag TB 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

Clinical specimen: Sputum, BAL, pus (abscess), blood, cell-free body fluids, urine and other respiratory specimens.
Bacterial culture in the solid and liquid media. Because the MTB is higly infectious agent, prepare sample in the BSC (Biosafety cabinet).

SAMPLE PREPARATION

Sputum/BAL or other respiratory specimen:

Method 1

- Liquify the sample*;
- Pellet by centrifugation at 12500 x g for 15 min;
- Discard supernatant, resuspend the pellet in 200 µl Buffer BL3, vortex mixing about 5 sec;
- Take 200 µl sample to sample tube for extraction.

Method 2 – Centrifuge free

- Liquify the sample*;
- Transfer 200 µl sample to sample tube;
- Add 200 µl buffer BL3 to sample (1:1).

** The liquefaction could be done by using liquefying agents, such as NALC (N-Acetyl-L-Cysteine) - NaOH and 0,75% DTT (5x stock) which could digest mucous material.*

Viscous body fluid (e.g. PUS**):**

- See procedure of **"Sputum/BAL or other respiratory specimen"**

Cell-free body fluid (e.g. CSF, urine)**:**

- Pellet bacteria by centrifugation at 14000 x g for 15 min;
- Discard supernatant, resuspend bacterial pellet in 200 µL Buffer BL3. Vortex-mixing about 5 sec;
- Take 200 µL sample to sample tube for extraction.

Liquefied, decontaminated sample:

- See the procedure of **"Cell-free body fluid"**

Blood or Blood-contaminated sample:

- Add cold sterilized water to sample to the ratio of water/blood about 3:1
- Inverted mix several times
- Incubate at 4°C, at least 10 min
- Centrifuge at 14000 x g for 15 min
- Remove supernatant, add 200 µL buffer BL3, vortex mixing about 5-10sec
- Take 200 µL sample to sample tube for extraction.

Colony from solid culture:

- Pick 1-3 colonies, mix with 200 µl Buffer BL3, vortex mixing about 5-10 sec;
- Take 200 µl sample to sample tube for extraction.

Liquid culture

Method 1

- Take 1 ml culture (>McFarland 0.5), transfer to 1,5 ml microcentrifuge tube;
- Pellet bacteria by centrifugation at 12500 x g for 5 min;
- Discard supernatant, resuspend bacterial pellet in 200 µl Buffer BL3, vortex mixing about 5-10 sec;
- Take 200 µl sample to sample tube for extraction.

Method 2 – centrifuge free

- Add Buffer BL3 to liquid culture (1:1);
- Vortex mixing about 5-10 sec;
- Take sample mixture to sample tube for extraction.

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 TB 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 TB DNA Extraction kit (code 2008).

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 TB 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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