SaMag Extraction kit User Manual

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

- **SaMag Tissue DNA Extraction Kit (SM004)**

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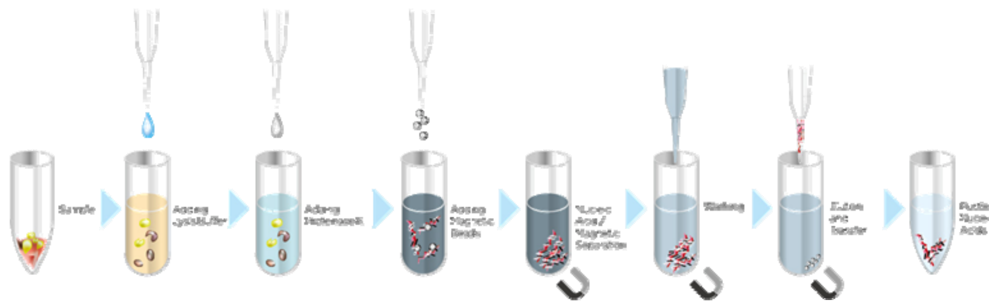
SaMag Tissue DNA Extraction Kit

NAME
SaMag Tissue DNA Extraction Kit

INTENDED USE
SaMag Tissue DNA Extraction Kit is designed to be used with SaMag-12/24 automatic nucleic acid extraction system for extraction of genomic DNA from a variety of animal tissues, swab samples and blood stain.

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);
- Proteinase K (10mg/ml), 1x1 ml
- Buffer BL2, 1 pc x 25 ml
- 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
- Shaking water bath or thermomixer

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	Empty	
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 Tissue 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

The types and amounts of starting material for use in SaMag Tissue DNA purification procedures are shown in Table listed below:

<table>
<tr><th>Sample Type</th><th>Target Nucleic Acid</th><th>Sample Volume
(Amount of starting material)</th><th>Elution Volume</th></tr>
<tr><td>Tissue</td><td rowspan="3">DNA</td><td>100-400 µl/10-40 mg</td><td rowspan="3">100-300µl</td></tr>
<tr><td>Dried swab samples (e.g. Buccal cells)</td><td>100-400 µl/1 swab or brush
(add BL2 and proteinase K to 100-400 µl for extraction)</td></tr>
<tr><td>Dried blood</td><td>100-400 µl/4 discs*</td></tr>
<tr><td>Control/Optional internal control</td><td colspan="3">Add controls/internal control in the extraction procedure if the downstream analysis needed.</td></tr>
</table>

**A 3mm diameter disc punched out from filter paper stained with dried blood contains white blood cells from approximately 5 µl whole blood; we recommend using 4 punched-out discs as starting material.*

YIELD OF PURIFIED DNA

DNA yields depend on the sample type, number of nucleated cells in the sample, and the protocol used for purification of DNA.
Table listed below shows DNA yields obtained from different sample types using SaMag Tissue DNA extraction procedures:

Sample Type	Sample Amount	Typical DNA Yield
Skeletal muscle	200 µl (40 mg tissue digested)	Up to 9µg
Heart	200 µl (20 mg tissue digested)	Up to 12µg
Spleen	200 µl (10 mg tissue digested)	Up to 27µg
Lung	200 µl (10 mg tissue digested)	Up to 17µg
Kidney	200 µl (10 mg tissue digested)	Up to 18µg
Liver	200 µl (10 mg tissue digested)	Up to 40µg
Buccal cells	1 Swab	1-5 µg
Duried blood	4 x 3 mm diameter discs	0.2-0.5 µg

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 steps below describe some recommendations for processing primary samples.

SAMPLE PREPARATION

For solid animal tissue:

<table>
<tr><td>1. Transfer tissue</td><td>Transfer tissue into a 1.5 ml microcentrifuge tube:
<table>
<tr><th>No.</th><th>Sample type</th><th>Recommended sample amount</th></tr>
<tr><td>1</td><td>Heart</td><td>20 mg</td></tr>
<tr><td>2</td><td>Muscle</td><td>40 mg</td></tr>
<tr><td>3</td><td>Other tissues</td><td>10 mg</td></tr>
</table></td></tr>
<tr><td>2. Add BL2 Buffer</td><td>Add 100-400 µl provided Buffer BL2. Ensure tissue pieces are fully submerged in Buffer BL2.</td></tr>
<tr><td>3. Add Proteinase K</td><td>Add 20 µl proteinase K solution and mix by vortexing.</td></tr>
<tr><td>4. Incubate</td><td>Incubate at 55°C in a shaking water bath or thermomixer (mix set at 1000r.p.m.)until the tissue is completely lysed.
Note: 1. Lysis time varies depending on the type of tissue processed. Lysis is usually completed in 1-2 hrs. However, lysis overnight is possible and does not influence the preparation. 2. Use the heat block for incubation, vortexing-mix several times during incubation is recommend.</td></tr>
<tr><td>5.Spin down and Transfer</td><td>Spin down and transfer 100-400µl the supernatant to Sample Tube. Use BL2 buffer to adjust the sample volume.
OPTIONAL: Using the filter column (not supplied in kit) to remove the residual debris and mucous material before DNA extraction will increase the DNA yield (20-100%).</td></tr>
</table>

For swab tissue:

1. Cut	Carefully cut or break off the end part of the swab or brush into a 2 ml screw-capped tube (not supplied) using an appropriate tool (e.g., scissors).
2. Add BL2 Buffer	Add 100-400 µl provided Buffer BL2 to the sample. Ensure sample is fully submerged in Buffer BL2
3. Add Proteinase K	Add 20 µl proteinase K, and mix thoroughly by vortexing for 10 s. If processing buccal cell brush samples, centrifuge the tube briefly (at 10,000 x g for 30s) to force the brush to the bottom of the tube.
4. Incubate	Incubate at 55°C for 15 min (place in a thermomixer, mix set at 1000 r.p.m. or vortex mix several times during incubation in heat block).
5.Centrifuge	Centrifuge the tube briefly to remove drops from inside the lid.
6. Remove	Remove the swab or brush from the tube. Using forceps, press the swab or brush against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately as the setting volume(100-400µl). *UseBL2 buffer to adjust the volume

Dried blood:

1. Collect	Collect 70 µl of each blood sample onto a ring marked on filter paper. Allow the blood to air dry. Either untreated blood or blood containing anticoagulant (EDTA, ACD, or heparin*) can be used. **heparin have inhibition effects on nucleic acid amplification reaction*
2. Cut	For each dried blood sample, use the manual paper punch to cut out four 3mm diameter discs.
3. Add BL2	Transfer each set of 4 discs to a 1.5 ml microcentrifuge tube. Add 220-440 µl BL2 to sample.
4. Add proteinase K	Add 20 µl proteinase K and mix by vortexing.
5.Incubation	Incubate at 55℃, 15 min in a thermomixer (set mix at 1000r.p.m.) or vortex mix several time during incubation in heat block.
6. Centrifuge	Centrifuge the tube briefly to remove the drop inside the lid.
7. Transfer	Transfer the 100-400 µl supernatant to the sample tube (provided in the kit), proceeding DNA 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 Tissue DNA Extraction kit Protocol, appropriate Starting Volume, Elution Volume

12 bis. In case of using SaMag-12 ver. 3.x EVO please use the touchscreen interface to select the Tissue DNA Extraction kit (code 2004).

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 Tissue 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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