SaMag Extraction kit User Manual

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

▪ SaMag Total RNA/DNA Extraction Kit (SM015)

Sample Type	Sample Volume (Amount of starting material)	Elution Volume
Whole blood	200-400 µl* (WBCs no. about 10^6)	50-200µl**
PBMCs	Up to 50 µl (suspended in 200 µl with RL buffer)	
Tissue	10-40mg (Lysed and suspend with RL buffer)	
Cultured cells	200-400 µl suspension of primary or cultured cells (cell no. < 5 x 106)	
Controls/internal control	Add controls /internal control in the extraction procedure if the downstream analysis is needed	

* Blood cells needs to perform manual RBC lysis procedure before extraction

** After extraction, store RNA/DNA at -60 to -80°C immediately, repeated freeze–thawing is not recommended

Sacace Biotechnologies Srl
via Scalabrini, 44 – 22100 – Como – Italy Tel +390314892927 Fax +390314492493
mail: ***info@sacace.com*** *web:* ***www.sacace.com***

IVD

REF SM015

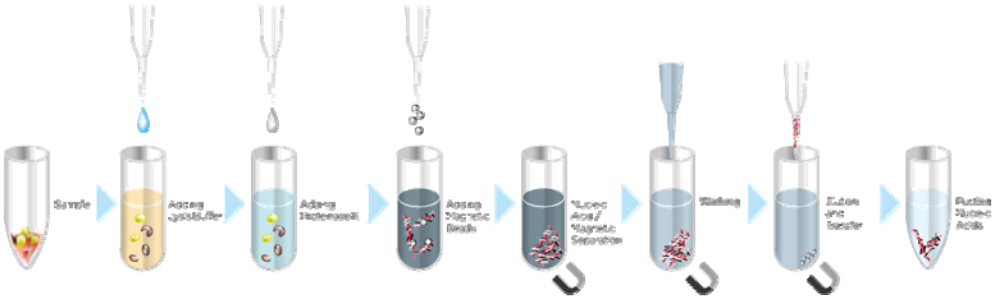
Σ 48

SaMag Total RNA/DNA Extraction Kit

NAME
SaMag Total RNA/DNA Extraction Kit

INTENDED USE
SaMag Total RNA/DNA Extraction Kit is designed to be used with SaMag-12/24 automatic nucleic acid extraction system for the extraction of Total RNA/DNA from whole blood, blood cells, animal tissue, tissue or cultured cells.

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);
- RL A Buffer, 25 ml;
- RL B Buffer, 25 ml;
- Filter Column, 50 pcs;
- Collection tube, 50 pcs;
- Barcode paper, 1 sheet;

Contains reagents for 48 tests.

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

REAGENTS REQUIRED BUT NOT PROVIDED

The reagents in the following table are required but not provided inside the Total RNA/DNA extraction kit:

Reagent	Description	Preparation
β -mercaptoethanol (β -ME)	β -ME reduce disulfide bonds and irreversibly denature the RNase and eliminate RNase released during cell lysis	Add 10 µl β-ME per 1 ml RL lysis Buffers*. It can be stored at RT for up to one month
Red blood cells lysis buffer	Lyse Erythrocyte from whole blood	10xRBC lysis buffer(100ml) 8.29g NH4Cl (1.5M)
(RBC lysis buffer)	(Erythrocyte (RBC) lysis procedure)	1g KHCO3 (100mM) 0.0372g Na2EDTA (10mM) Adjust pH7.2-7.4 by HCl 0.2 mm filtered, store for 6 months at 4°C Dilute 10 times fresh before use
DNase	To eliminate DNA contamination	Novagen RNase-free DNaseI (69182-3CN)
10x DNase buffer	To eliminate DNA contamination	0.5M Tris-HCl 25mM MgCl2 5mM CaCl2

*RL lysis buffers means RLA and RLB Buffers. Dispense the β-ME in a fume hood and wear appropriate protective clothing.

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 1	720 µl
well-3	Binding Buffer 1	1000 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	RNase-free water	1000 µl
well-9	Rnase-free water	1000 µl
well-10	DNase buffer	1000 µl

STORAGE

SaMag Total RNA/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.
After extraction, store RNA/DNA at -60 to -80 °C immediately, repeated freeze–thawing is not recommended. Always handle RNA/DNA on ice for downstream analysis. Store the RL A and RL B buffers at 2-8°C.

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.

SPECIMEN COLLECTION

Whole Blood

1. Use fresh whole blood sample for isolation collected within 4 hr, on ice. Freezing blood is not recommended. The blood sample should be collected in the presence of an anticoagulant, preferably EDTA, although other anticoagulants such as citrate or ACD (acid citrate dextrose) can also be used.
2. For optimal results, blood samples should be processed within a few hours from collection and kept at 2-8°C.
3. Perform Erythrocyte (RBC) lysis procedure before extraction.
4. When using whole blood samples which have extreme high WBCs no. (more than 10000/µl) or concentrated PBMCs (peripheral blood mononucleated cells), decrease of the input volume for extraction is recommended (total WBC no. less than 5 x 10^6).

Tissue

1. To prevent degradation by intracellular RNase, it is important that tissues are either flash-frozen in liquid nitrogen and stored at –70°C, or processed immediately following excision.
2. Use RNA stabilizing reagent (e.g. RNA later) to treat tissue is another option to protect the RNA if the sample cannot be frozen immediately. Frozen tissue should not be allowed to thaw during handling (e.g., weighing), keeping the sample on ice during cutting or homogenizing with RLA Buffer is recommended.
3. After homogenization, use filter column (supplied in the kit) to remove the insoluble and viscous material of the lysates.

Cells

1. Cells or isolated blood cells can be collected as pellets and either flash-frozen in liquid nitrogen and stored at –70°C, or processed immediately. Add RLA Buffer to resuspend pellet for extraction
2. Alternatively, samples can be stored at –70°C in RLA Buffer after disruption and homogenization. Samples frozen in this way are stable for months.

NOTE: it is essential to use the correct amount of starting material in order to obtain optimal RNA/DNA yield and purity (look at table below). Use excess quantity is not helpful in Total RNA/DNA extraction.

STARTING MATERIAL

Sample Type	Sample Volume (Amount of starting material)	Elution Volume
Whole blood	200-400 µl* (WBCs no. about 10^6)	50-200µl**
PBMCs	Up to 50 µl (suspended in 200 µl with RL buffer)	
Tissue	10-40mg (Lysed and suspend with RL buffer)	
Cultured cells	200-400 µl suspension of primary or cultured cells (cell no. < 5 x 10^6)	
Controls/internal control	Add controls /internal control in the extraction procedure if the downstream analysis needed	

* Blood cells needs to perform manual RBC lysis procedure before extraction
** After extraction, store RNA/DNA at -60 to -80°C immediately, repeated freeze–thawing cycles are not recommended

PRETREATMENT (SAMPLE PREPARATION)

Sample	Procedure
Whole blood	1. Prepare a fresh 1x RBC lysis buffer 2. Add ice-cold two volume RBC lysis buffer to one volume blood sample 3. Inverting 3-5 times, incubate on ice for 10-15 min 4. Centrifuge at 1000 x g, 10min, 4°C 5. Remove supernatant 6. Resuspend pellet with 220 µl RLA Buffer 7. Take 200 µl for extraction
PBMCs (Peripheral Blood Mononucleated Cells)	1. Resuspend PBMCs with 220 µl RLA Buffer 2. Vortex mixing for 10 sec 3. Take 200 µl for extraction
Tissue	1. Add 220 µl RLA Buffer to tissue; make sure the sample if completely immersed in buffer. Increase RLA buffer input amount up to 440 µl if tissue sample is large. 2. Homogenize tissue by homogenizer 3. Spin down the lysate 4. Remove all the lysate to filter column sitting in collection tube 5. Centrifuge at 1000 x g , for 5min on 4°C 6. Transfer 200-400 µl to sample tube 7. Perform extraction

Cultured cells	(Protocol 1) Suspension culture 1. Harvest cell culture 2. Centrifuge at 1000xg, 5min, 4°C 3. Remove supernatant completely 4. Resuspend cell pellet with 220 µl RLA Buffer 5. Vortex mixing for 10 sec 6. Take 200 µl for extraction (Protocol 2-1) Monolayer culture 1. Trypsinize the cells 2. Harvest the cell in PBS 3. centrifuge at 300xg, 5min, 4°C 4. Remove supernatant 5. Resuspend pellet with 220 µl RLA Buffer 6. Vortex mixing for 10sec 7. Take 200 µl for extraction (Protocol 2-2) Monolayer culture 1. Scrape the cells with 220-440 µl RLA Buffer 2. Vortex mixing for 10sec 3. Take 200-400 µl for extraction
DNA-free RNA extraction	1. After Total RNA/DNA program extraction 2. Add 2U DNase I (not provided) to the eluate 3. Incubate at 37°C, 10min 4. Transfer mixture to a new sample tube 5. Proceed with new “Total RNA/DNA ” protocol to start extraction

PROTOCOL

To perform extraction start SaMag-12/24 instrument, open door(s) and follow steps indicated in SaMag user manual in chapter 2.3 “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 Total RNA/DNA Extraction kit Protocol, appropriate Starting Volume, Elution tube used, Elution Volume (suggested values are 200 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 Total RNA Extraction kit (code 2015).

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

*** 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.**

Total RNA/DNA extracted with SaMag Total RNA/DNA Extraction Kit must be stored at -60 to -80°C immediately, repeated freeze–thawing cycles are not recommended.

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