OxMag Blood and Tissue DNA Purification Kit

High-Throughput Technology

Qualitative Assay for Automatic Extraction Systems

INSTRUCTION FOR USE

Version 3. MAG-006.11.23

RUO

Research Use Only

REF

MAG-006

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EC REP

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List of content

Description of the kit

Intended use

The OxMag Blood and Tissue DNA Purification Kit is designed for the efficient isolation of DNA from buffy coat, whole blood, solid tissue (semi-solid bone marrow) and cell suspension /cell cultures (including HeLa cells). It is especially suited for DNA extraction from human and animal samples. The technology allows high-throughput analyses, requires low handling, gives an advantage in simplicity and speed, reduces the risk of contamination. The analytical performance and sensitivity of the kit were thoroughly tested in laboratory and clinical settings according to the "EU Regulation 2017/746 on In Vitro Diagnostic Medical Devices."

The kit components

- The kit composition is described in "**Table 1. The buffers and components of the kit."**
- Instructions for Use (IFU) – detailed guidelines for high-quality DNA Purification methodology which could be easily adapted to the various automated systems and samples.
- Material Safety Data Sheet (MSDS) – the documents contain information about hazard identification, first aid measures, firefighting, accidental release measures, handling and storage, exposure control, and personal protection.

Table 1. The buffers and components of the kit

OxMag Pathogen DNA/RNA Purification Kit		200 rxn	500 rxn	2,000 rxn	Transportation and Storage
Solution A	Lysis Buffer	40 ml	100 ml	400 ml	Room temperature
Solution B	Lysis/Binding Buffer	61 ml	80 ml x 2	121 ml x 5	Room temperature
Solution W1 (conc.)	Wash Buffer 1	30 ml	75 ml	295 ml	Room temperature
Solution W2 (conc.)	Wash Buffer 2	14 ml x 2	70 ml	140 ml x 2	Room temperature
Solution E	Elution Buffer	50 ml	105 ml	105 ml x 4	Room temperature
OxMag® Beads* *Magnetite*	Synthetic Magnetic Beads	4 ml	10 ml	40 ml	Room temperature
Proteinase K	Enzyme *Lyophilized Powder*	40 mg x 2	40 mg x 5	40 mg x 20	Room temperature
RNase A	Enzyme *Lyophilized Powder*	8 mg	10 mg x 2	10 mg x 8	Room temperature
Proteinase K Storage Buffer	Storage Buffer for Enzyme	1.5 ml x 2	6 ml	25 ml	Room temperature
RNase A Storage Buffer	Storage Buffer for Enzyme	1.5 ml	1.5 ml x 2	9 ml	Room temperature

* - Do not freeze OxMag® Beads solution. Store at 2-8 ºC

Reagents Preparation

Solution W1

Wash Buffer 1 comes as a concentrate. Prior to initial use, combine the recommended quantity of ethanol, which must be at least 95% pure, as specified in "Table 2. Preparation of Solution Wash 1"

Table 2. Preparation of Solution Wash 1

No. Reactions	Solution W1	Ethanol ≥95%	Final Volume
200	30 ml	75 ml	105 ml
500	75 ml	180 ml	255 ml
2000	295 ml	705 ml	1,010 ml

Solution W2

Wash Buffer 2 comes as a concentrate. Prior to initial use, combine the recommended quantity of ethanol, which must be at least 95% pure, as specified in "Table 3. Preparation of Solution Wash 2"

Table 3. Preparation of Solution Wash 2

No. Reactions	Solution W2	Ethanol ≥95%	Final Volume
200	14 ml x 2	71 ml (In each bottle)	85 ml x 2 bottles
500	70 ml	350 ml	420 ml
2000	140 ml x 2	700 ml (In each bottle)	840 ml x 2 bottles

Proteinase K

Proteinase K is supplied as a lyophilized powder. Before using it for the first time, add the appropriate amount of Proteinase K Storage Buffer, as indicated in "Table 4. Preparation of Proteinase K enzyme". Aliquoted Proteinase K should be stored at -20 °C.

Table 4. Preparation of Proteinase K enzyme

No. Reactions	Proteinase K	Proteinase K Storage Buffer	Final Volume
200	40 mg x 2	1 ml (In each vial)	1 ml x 2 vials
500	40 mg x 5	1 ml (In each vial)	1 ml x 5 vials
2000	40 mg x 20	1 ml (In each vial)	1 ml x 20 vials

RNase A

RNase A is supplied as a lyophilized powder. Before using it for the first time, add the appropriate amount of RNase A Storage Buffer, as indicated in "Table 5. Preparation of RNase A enzyme". Aliquoted RNase A should be stored at -20 °C.

Table 5. Preparation of RNase A enzyme

No. Reactions	Solution B	OxMag® Beads	Final Volume
200	8 mg	0.8 ml	0.8 ml x 1 vial
500	10 mg x 2	1 ml (In each vial)	1 ml x 2 vials
2000	10 mg x 8	1 ml (In each vial)	1 ml x 8 vials

Lysis/Binding Bead Mix (For Routine Testing)

Prepare Solution B with Magnetic Beads for routine testing. Add the appropriate volume of components indicated in "Table 6. Preparation of Solution B with Magnetic Beads" for same-day use.

Table 6. Preparation of Solution B with Magnetic Beads

No. Reactions	Solution B	OxMag® Beads	Final Volume
200	61 ml	4 ml	65 ml
500	80 ml x 2	5 ml (In each bottle)	85 ml x 2
2000	121 ml x 5	8 ml (In each botte)	129 ml x 5

Recommended Sample Pretreatment

Buffy coat

To prepare buffy coat, centrifuge whole blood with an anticoagulant (EDTA, citrate, or heparin) at 900 – 1,100 × g for 10 minutes at room temperature. From 10 ml of centrifuged blood, harvest approximately 1 ml of the leukocyte-rich fraction, achieving a 5 – 6 × enrichment. For instance, whole blood with a white blood cell count of 6×10^6 cells/ml yields buffy coat with ~3×10^7 cells/ml. A protocol using 200 µl buffy coat will therefore utilize 0.6×10^7 cells.

Whole blood

Before processing, ensure that entire blood samples are fully liquefied to prevent clot carryover, which could interfere with nucleic acid purification. Blood can be stored with anticoagulants (EDTA, citrated, heparin).

Solid Tissue

Take up 25-50 mg tissue (up to 10 mg spleen or liver) and place in a 1.5 ml microfuge tube. Add 190 µl of Solution A and 10 µl of Proteinase K. Mix thoroughly by bead beating machine or homogenizer depending on sample type. Spin down the tube to remove drops from inside the lid.

Bone marrow samples must be processed under sterile conditions to maintain the integrity of nucleic acids. If collected in an anticoagulant tube (e.g., EDTA, citrate), the sample should be mixed gently to avoid clot formation. Washing the sample with phosphate-buffered saline (PBS) may be performed to eliminate plasma and cellular debris.

Cell suspension /cell culture

Before DNA extraction, treat cell cultures to ensure high-quality nucleic acids. Wash cells twice with PBS to remove media and inhibitors. Process the pellet immediately or store at -80°C. Handle carefully under sterile conditions, avoiding freeze-thaw cycles to preserve DNA integrity.

The List of Materials to be Supplied by the User

Table 7. Equipment and Reagents to be Supplied by the User

Equipment	Consumables
Thermoblock or thermomixer	Ethanol ≥95%
Centrifuge	OxBeads zirconia/steel beads (Optional)
Bead Beating Machine and/or vortex	RNase-free 1.5 ml microcentrifuge tubes
Pipette 0.5 - 10 µl	Benchtop cooler or ice box
Pipette 20 - 200 µl	0.5 - 10 µl pipette tips with filter
Pipette 100 - 1000 µl	20 – 200 µl pipette tips with filter
Magnetic rack	100 - 1000 µl pipette tips with filter
Tecan, King Fisher Flex or other liquid handlers	Automation platform-compatible plastics

Instructions for Automated Purifications

DNA isolation from blood

The sample should be stored according to "Interim Guidelines for Collecting and Handling of Clinical Specimens (Center for Disease Control, CDC)". Before analysis, ensure the samples do not contain clots and sediments.

Before starting the procedure, prepare the solutions and enzymes according to the solution preparation guide (Tables 2-6). **Do not freeze OxMag® Beads solution.** Store at 2-8 ºC. Before pipetting, ensure that the OxMag® Beads solution is homogeneous to have an even distribution of nanoparticles (use a vortex). Solution B may form precipitates upon storage. Warm them up to 60°C until the residues have fully dissolved.

1. Set up the instrument (200 µl sample input volume).

2. Prepare Solution B with Magnetic Beads. For one reaction, add 20 µl of OxMag® Beads to 300 µl Solution B (Lysis/Binding buffer). For routing testing, use Table 6.

3. Prepare the processing plates:

- Plate wash (plate position 2): Add 500 µl of Solution W1 to each well in the plate;
- Note: RNase I treatment optional but recommended. RNase I is not supplied with the kit. Plate wash (plate position 3): Add 800 µl of Solution W2 to each well in the plate;
- Plate elution (plate position 4): Add 50-200 µl of solution E to each well in the plate.

4. Prepare sample plate:

- Add 10 µl of Proteinase K to each sample well;
- Add 200 µl of sample to each sample well;
- Add 200 µl of Nuclease-free Water to the Negative Control well.

Invert Solution B mixed with Magnetic Beads gently to have a homogeneous mixture, then add 320 µl to each well. Note: If RNA-free genomic DNA is required, add RNase 4 µl to each sample well.

5. Load the prepared plates into the appliance according to the relevant appliance.

DNA isolation from solid tissue samples

Before starting the procedure, prepare the solutions and enzymes according to the solution preparation guide (Tables 2-5). **Do not freeze OxMag® Beads solution** and store at 2-8 ºC. Before pipetting, ensure that the OxMag® Beads solution is homogeneous and has an even distribution of nanoparticles (use a vortex). Solution A and Solution B may form precipitates upon storage. Warm them up to 60°C until the residues have fully dissolved.

1. Cut up to 25-50 mg tissue (up to 10 mg spleen or liver) into small pieces on ice, place in a 1.5 ml microfuge tube or use OxBeads zirconia/steel beads (not supplied by the manufacturer). Add 190 µl of Solution A and 10 µl of Proteinase K. Mix thoroughly by bead beating machine or high-speed homogenizer for 2-5 min. Spin down the tube to remove drops from inside the lid.

2. Incubate for 2-24 hours at 56°C in a thermomixer with full-speed agitation (1,400 rpm), or incubate in a thermo-block, vortexing the samples every 30 min.

3. Centrifuge the sample for 2 min at 13,000 rpm and transfer 200 µl of supernatant to a new micro-centrifuge tube. A thin layer will be formed on top of the clean supernatant. Carefully collect only the clean supernatant, although small pieces of the layer will not affect the quality of the DNA.

4. Set up the instrument (200 µl sample input volume). Prepare Solution B with Magnetic Beads. For one reaction, add 20 µl of OxMag® Beads to 300 µl Solution B (Lysis/Binding buffer). For routing testing, use Table 5.

5. Prepare the processing plates:

- Plate wash (plate position 2): Add 500 µl of Solution W1 to each well in the plate;
- Note: RNase I treatment optional but recommended. RNase I is not supplied with the kit. Plate wash (plate position 3): Add 800 µl of Solution W2 to each well in the plate;
- Plate elution (plate position 4): Add 50-200 µl of solution E to each well in the plate.

6. Prepare sample plate:

- Add 200 µl of lysate to each sample well;
- Add 200 µl of Nuclease-free Water to the Negative Control well;
- Invert the Binding Bead Mix gently to have a homogeneous mixture, then add 320 µl to each well.

7. Load the prepared plates into the instrument according to the relevant instructions

Disposal

Dispose of used kit reagents, human clinical samples, and sealed amplification plates as laboratory clinical waste according to local, state, and federal regulations.

Version History

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Quality Control System

The Kit is in accordance with 2017/746-EN Medical Device Regulations.

Technical support

For technical support, please contact our dedicated Technical Support Team at:

TEL: +995 599 374 374, Email: info@oxgen.ge

Trademarks and Disclaimers

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Explanation of Symbols

	Attention
	Lot Number
	Catalogue Number
	Production Date
	Refer to the Operating Instructions
	Shelf life
	If the Package Is Damaged “Do Not Use It”
	Manufacturer Information
	EC Representative
	Temperature limit