

OxMag RNA Purification Kit

High-Throughput Technology

Qualitative Assay for Automatic Extraction Systems

INSTRUCTION FOR USE



Version 3. MAG-017.11.23

RUO

Research Use Only

REF

MAG-017



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Description of the kit

Intended use

OxMag RNA Purification Kit is a universal magnetic bead-based product for rapid and reliable isolation of RNA from buffy coats, whole blood, cell suspensions and other liquid samples¹. 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 RNA 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 RNA Purification Kit		200 rxn	500 rxn	2,000 rxn	Transportation and Storage
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* <i>Magnetite</i>	Synthetic Magnetic Beads	4 ml	10 ml	40 ml	Room temperature
Proteinase K	Enzyme <i>Lyophilized Powder</i>	40 mg x 2	40 mg x 5	40 mg x 20	Room temperature
Proteinase K Storage Buffer	Storage Buffer for Enzyme	1.5 ml x 2	6 ml	25 ml	Room temperature
DNase I	Enzyme	1 ml	1.25 ml x 2	1 ml x10	Room temperature
DNase I Activation Buffer	Activation buffer for Enzyme	16 ml	38 ml	76 ml x 2	Room temperature

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

¹ The kit can be extended towards veterinary and zoonotic research and diagnostics.

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 $\geq 95\%$	Final Volume
200	30 ml	75 ml	105 ml
500	75 ml	180 ml	255 ml
2000	295 ml	715 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 $\geq 95\%$	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 $-20\text{ }^{\circ}\text{C}$.

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

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 5. Preparation of Solution B with Magnetic Beads” for same-day use.

Table 5. 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

DNase I mix with activation buffer

DNase I is supplied in storage buffer. For same day use add the appropriate amount of DNase I Activation Buffer, as indicated in “Table 6. Preparation of DNase I enzyme” for same-day use.

Table 6. Preparation of DNase I enzyme

No. Reactions	DNase I	DNase I Activation Buffer	Final Volume
1	5 µl	75 µl	80 µl
96	480 µl	7.2 ml	7.68 ml
200	1 ml	15 ml	16 ml
500	2.5 ml	37.5 ml	40 ml

Recommended Sample Pretreatment

Buffy coats

To prepare buffy coat, centrifuge whole blood with an anticoagulant (EDTA, citrate, or heparin) at 900–1100 × 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 × 10⁶ cells/ml yields buffy coat with ~3 × 10⁷ cells/ml. A protocol using 200 µl buffy coat will therefore utilize 0.6 × 10⁷ 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).

Cell suspension

Before RNA extraction, treat cell cultures to ensure high-quality nucleic acids. Wash cells twice with PBS by centrifugation to remove media and inhibitors and dissolve pellet in 200 µl PBS. Process the pellet immediately, handle carefully under sterile conditions, avoiding freeze-thaw cycles to preserve RNA integrity. For solid tissues use mechanical homogenization and OxBeads zirconia/steel beads by beating machine.

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	RNase-free 1.5 ml micro-centrifuge 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

RNA isolation from liquid samples

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 inhibiting mucus 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 °C. Before pipetting, ensure that the OxMag® Beads solution is homogeneous and has 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 5.
3. Prepare the processing plates:
 - Plate wash (plate position 2): Add 500 µl of Solution W1 to each well in the plate;
 - **Note: DNase I treatment optional but recommended.** Plate DNase I treatment (plate position 3): Add 80 µl of premixed DNase I enzyme, incubate for 20 min;
 - Plate wash (plate position 4): Add 800 µl of Solution W2 to each well in the plate;
 - Plate elution (plate position 5): 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.

5. Load the prepared plates into the instruments according to the relevant instructions.

Troubleshooting RNA purification

Table 8. Possible causes and solutions for problems that may occur during RNA preparation

Problem	Possible cause	Solution
Agglomerated Magnetic Nanoparticle Solution	Freezing of Magnetic Nanoparticle Solution	Frozen Magnetic Nanoparticles cannot be recovered resulting in non-valid outcomes
Low yield and/or quality RNA	Problems with sample storage and handling	Endogenous RNases can cause the degradation of RNA. It is important to follow the proper guidelines for storing and handling the sample to avoid degradation
	RNases were introduced during/after sample processing.	Make sure to wear gloves at all times and to change them frequently. Follow good laboratory practice for treating glassware, plastic ware, and solutions that will come into contact with RNA
	The sample was not disrupted/homogenized thoroughly	Follow disruption/homogenization recommendations for your sample type
Inhibition of downstream application	Problems with sample amount and elution step	Dilute the samples, increase or decrease the amount of Elution buffer
	High salt concentrations or the presence of contaminants	Follow the proper guidelines for Wash and Elution Step

Precautions for Users




- OxMag Kit is intended to be used by laboratory and healthcare professionals. It is not intended for self-testing and near patient testing.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
- Handle all biological samples as potentially infectious and avoid direct contact.
- Follow relevant national biosafety regulations and WHO and CDC guidelines for specimen collection and processing.
- Trained professionals should only use the kit in a diagnostic or research laboratory with appropriate equipment and safety standards.
- Review the safety data sheets (MSDS) provided with the kit.
- Use designated laboratory equipment, consumables, and personal protective equipment in each room.
- Follow Good Laboratory Practices by wearing protective clothing, gloves, goggles, and a mask. Avoid eating, drinking, smoking, or applying cosmetics in the working area.

- Do not mix reagents from different sources or kits.
- Clean equipment and surfaces regularly with appropriate bleach concentration or equivalent decontaminants that remove nucleic acids, followed by 70% ethanol.

Chemical Safety

- The components of the OxMag Viral RNA purification kit with hazardous content and handling instructions are summarized in “Table 9. Hazardous content and handling instructions”. Wear gloves, goggles and protective equipment and follow the safety instructions given in the table.

Table 9. Hazardous content and handling instructions

Component	GHS symbol	Category	Statements
Solution B	 GHS05, GHS07	Acute Toxicity (Oral) – Category 4 SKIN CORROSION/IRRITATION - Category 2 SERIOUS EYE DAMAGE/EYE IRRITATION – CATEGORY 2 A	Hazard Statements: H302+H332, H315, H19 Precautionary Statements: P260, P273, P280, P303 + P353, P306 + P361 + P353, P304 + P340 + P312, P305 + P351 + P338 + P310
Solution W 1 and W2	 GHS02, GHS06, GHS08	Acute Toxicity (Oral) – Category 4 SKIN CORROSION/IRRITATION - Category 2 SERIOUS EYE DAMAGE/EYE IRRITATION –CATEGORY 2 A	Hazard Statements: H225, H301 + H311 + H331, H370 Precautionary Statements: P210, P280, P301 + P310 + P330, P302 + P352 + P312, P304 + P340 + P311
Proteinase K	 GHS07, GHS08	SKIN IRRITATION, Category 2 EYE IRRITATION, Category 2 RESPIRATORY SENSITIZATION - Category 1	Hazard Statements: H315, H17, H19, H334, H335 Precautionary Statements: P260, P285, P304 + P341, P342 + P311

Hazard Statements

H302+H332	Harmful if swallowed, Harmful if inhaled
H315	Causes skin irritation
H317	May cause an allergic skin reaction
H319	Causes serious eye irritation
H225	Highly Flammable liquid and vapor
H301+H311+	Toxic if swallowed, in contact with skin or if inhaled
H331	
H319	Causes serious eye irritation
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled
H335	May cause respiratory irritation

Precaution Statements

P210	Keep away from heat, hot surface, sparks, open flames and other ignition sources. - No smoking.
P260	Do not breathe dust/fume/gas/mist/vapors/spray.
P273	Avoid release to the environment.

P280	Wear protective gloves/protective clothing/eye protection/face protection.
P285	In case of inadequate ventilation wear respiratory protection
P301 + P310 + P330	IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Rinse mouth.
P302 + P352 + P312	IF ON SKIN: Wash with plenty of water. Call a POISON CENTER or doctor if you feel unwell.
P303 + P353	IF ON SKIN (or hair): Rinse skin with water [or shower].
P304 + P340 + P311	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a POISON CENTER or doctor.
P304 + P340 + P312	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a POISON CENTER or doctor if you feel unwell.
P304 + P341	IF INHALED: If breathing is difficult, remove person to fresh air and keep comfortable for breathing.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/physician.
P306 + P361 + P353	IF ON CLOTHING: Take off immediately all contaminated clothing.
P342 + P311	If experiencing respiratory symptoms: Call a POISON CENTER/ doctor.

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

Instruction for Use Version 3. MAG-017. EN. Nov 2023.

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

OxMag is a trademark of OxGEN, LLC. AM 2021 11324.

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

