MO BIO Laboratories, Inc.

Saving You Time For Life

PowerMag® Blood DNA/RNA Isolation Kit

(Optimized for KingFisher®)

Catalog No. 22100-4-KF

Quantity: 4 x 96 Preps (Flex) or 32 x 12 Preps (Duo)
Total Purifications: 384

INSTRUCTION MANUAL

Version 10292014

Please recycle

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TABLE OF CONTENTS

KIT CONTENTS

	Kit Catalog #22100-4-KF	
Component	**Catalog#**	**Amount**
PowerMag® RBC Lysis Solution	22100-4-KF-1	255 ml
PowerMag® WBC Lysis Solution	22100-4-KF-2	220 ml
ClearMag® Binding Solution	22100-4-KF-3	200 ml
ClearMag® Beads	22100-4-KF-4	9 ml
ClearMag® Wash Solution	22100-4-KF-5	640 ml
ClearMag® RNase-Free Water	22100-4-KF-6	43 ml
MO BIO 2 mL Deep Well Plates (DWP)	22100-4-KF-DWP	4 plates
Sealing Tape	22100-4-KF-ST	8 tapes

KIT STORAGE

The kit reagents and components should be stored at room temperature (15-30°C).

PRECAUTIONS

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Safety Data Sheets (SDS) for emergency procedures in case of accidental ingestion or contact. All SDS information is available upon request (760-929-9911) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

Please follow Universal Precautions when working with whole blood and/or blood products to prevent exposure to bloodborne pathogens.

This kit is for research purposes only. Not for diagnostic use.

REQUIRED BUT NOT INCLUDED

Equipment

- ☐ Centrifuge capable of handling two 96 Well Blocks (13 cm x 8.5 cm x 6.0 cm) at 2000 x g
- ☐ Multi-channel pipettor(s) (volumes of 10 µl - 900 µl)
- ☐ Single pipettor(s) (volumes of 10 µl – 1000 µl)
- ☐ Orbital plate shaker capable of 450 rpm

Reagents

- ☐ ß-mercaptoethanol (ß-ME) (480 µl of ß-ME is required for a single full 96-well plate).
- ☐ 100% Ethanol is required for the wash steps in the protocol (190 ml of 100% ethanol is required for a single full 96-well plate).

Consumables

- ☐ Contact your Thermo Scientific representative for the KingFisher® Flex and Duo consumables specific to your platform. Go to www.mobio.com/powermag for links to the necessary KingFisher® products on the ThermoFisher Scientific website.
- ☐ Reagent reservoirs for 5 – 300 ml volumes.
- ☐ Pipette tips (volumes of 10 µl - 900 µl) for single use pipettes and multi-channel pipettor(s) to be used in the lysate preparation steps.

PROTOCOL OVERVIEW

PowerMag® Blood DNA/RNA Isolation Kit

(Optimized for KingFisher®)
Catalog No. 22100-4-KF

The PowerMag® Blood DNA/RNA Kit is optimized for use with the Thermo Scientific KingFisher® Flex and KingFisher® Duo platforms.

The PowerMag® Blood DNA/RNA Kit can be used for automated, high throughput, and simultaneous co-isolation of DNA and RNA from 200 µl of anti-coagulant (EDTA and heparin) stabilized whole blood. This protocol has been validated with fresh blood (including blood stored in commercial preservation solutions, see Hints and Troubleshooting Guide) and can isolate DNA from buffy coats as well as frozen blood. Use of MO BIO's novel ClearMag® magnetic beads enables efficient co-extraction of DNA and RNA from the same sample resulting in high yields, excellent purity, and high molecular weight DNA (up to 100 kb). Furthermore, ClearMag® bead technology provides a robust method for preventing the co-isolation of common blood-derived PCR inhibitors, thereby ensuring successful downstream applications such as PCR and RT-PCR.

200 µl of whole (or preserved) blood is added to a MO BIO 2 ml Deep Well Plate (DWP). After aliquoting blood samples into the plate, the protocol begins with erythrocyte lysis using a hypotonic buffer that enables removal of Red Blood Cells (RBCs). After centrifugation, nucleic acids are extracted from the remaining White Blood Cell (WBC) pellet using a highly denaturing chaotropic buffer that stabilizes and protects nucleic acids from liberated nucleases.

Once lysates have been generated, they are combined with ClearMag® Beads, transferred to the KingFisher® instrument and the remaining protocol steps are performed fully automated with DNA and RNA being co-eluted in RNase-Free Water.

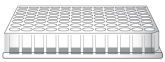
Note

The order of placement of components and reagents for the platform portion of the protocol will be described in the downloaded software specific to your KingFisher® platform.

Other open platform robots may be used with this kit. However, you may need to contact your local field application scientist or the manufacturer of your robot for help in adapting this protocol to your system.

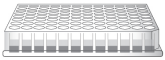
PowerMag® Blood DNA/RNA Isolation Kit

Prepare Sample

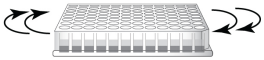


- Add 200 µl of blood to a MO BIO 2 mL Deep Well Plate (DWP)

RBC Lysis

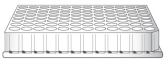


- Add 600 µl PowerMag® RBC Lysis Solution



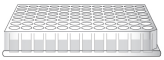
- Place plate on an orbital plate shaker and shake for 10 min

- *Centrifuge @ 2000g for 5 min*

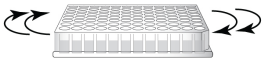


- Remove 750 µl of the supernatant

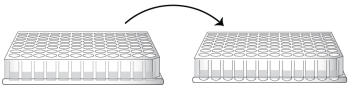
WBC Lysis



- Add 500 µl PowerMag® WBC Lysis Solution

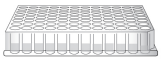


- Place plate on an orbital plate shaker and shake for 10 min



- Transfer the supernatant to a clean KingFisher® Microtiter Deep Well 96 Plate

Follow KingFisher® platform specific protocol for next steps



- Add 20 µl of ClearMag® Beads
- Add 410 µl of ClearMag® Binding Solution
- Add 900 µl of ClearMag® Wash Solution
- Add 900 µl of 100% Ethanol
- Add 50-100 µl of ClearMag® RNase-Free Water

Place KingFisher® Microtiter Deep Well 96 Plate on the KingFisher® platform and initiate the PowerMag® Blood protocol

EXPERIENCED USER PROTOCOL

Please wear gloves and follow Universal Precautions at all times.

Important Notes Before Starting:

- **Not all blood samples are the same. They differ in the number of WBCs and this affects the quantity of nucleic acids isolated from each sample. WBC count can vary based on the health of the subject at the time of blood sampling. Nucleic acid yields vary by species as well**.
- An important consideration for extraction of RNA from blood is the handling and storage of samples. Blood should be collected in tubes containing an anticoagulant such as EDTA, ACD, CPD-A or heparin. **Extractions should be performed as soon as possible (within 2 hours) after blood collection to obtain the highest quality and yields of DNA and RNA.** Freezing of the samples should be avoided, particularly in applications where RNA is desired unless a validated DNA/RNA stabilizer such as PAXgene™ Tubes (www.preanalytix.com) or Biomatrica RNAgard® Blood System (www.biomatrica.com/rnagardblood_tube.php) is used. Always use fresh blood for optimal results.
- You will need 190 ml of 100% Ethanol for each full 96 well plate being processed on the KingFisher® Flex. The KingFisher® Duo requires 22 ml for 12 wells to be processed.

Prepare PowerMag® WBC Lysis Solution by adding ß –mercaptoethanol (ß-ME)

- For each full 96 well plate being processed, prepare a fresh WBC lysis buffer solution consisting of 49.5 ml **PowerMag® WBC Lysis Solution** and 500 µl ß-ME (1% v/v, 1:100 dilution). One sample requires 5 µl of **ß-ME** + 495 µl of **PowerMag® WBC Lysis Solution**.

Note

Prepare PowerMag® WBC Lysis Solution in smaller aliquots with fresh ß-ME according to the number of samples you need to process that day instead of adding ß -ME to the whole bottle.

1. Dispense 200 µl of well-mixed, fresh blood into each well of the **MO BIO 2 ml Deep Well Plate (DWP)**. (For preserved blood, please see Hints and Troubleshooting guide.)

2. Add 600 µl of **PowerMag® RBC** to each well and mix the sample by repeated pipetting (3 – 5 times). Apply a piece of Sealing Tape (provided) to the top of the 96 well plate. Place on an orbital plate shaker and shake at 450 rpm for 10 minutes at room temperature to lyse the red blood cells (RBCs).

3. Centrifuge the **DWP** at 2000 x g for 5 minutes to pellet the intact white blood cells (WBCs). Please remember to prepare a balance plate for the centrifugation step; use 800 µl of water for each well. Remove sealing tape and discard.

4. Remove and discard 750 µl of RBC lysate from each well. We recommend inserting your pipette tip along the wall of the well to avoid disturbing the WBC pellet at the center of the well floor.

Note

If using a digital pipettor to remove the RBC lysate, we recommend setting the aspiration speed on the lowest setting to avoid disturbing the WBC pellet during aspiration.

5. Add 500 µl of **PowerMag® WBC Lysis Solution/ß-ME** (see Important Notes Before Starting) to each well and re-suspend the WBC pellet by pipetting up and down 3 to 5 times. Apply a new piece of Sealing Tape (provided).

6. Place on a plate shaker and shake at 450 rpm for 10 minutes at room temperature.

7. Open the appropriate protocol on your instrument specific to your platform and then proceed. For KingFisher® Flex applications see page 13, and for KingFisher® Duo go to page 14.

KingFisher® Flex Protocol (continued from step 7 on page 12)

See Consumables on page 7 for the list of necessary user-supplied materials.

8. Transfer entire WBC lysate (from step 6, approx. 550 µl) to the appropriate wells on a KingFisher® Microtiter Deep Well 96 Plate (user supplied).

9. For each plate to be processed, resuspend the **ClearMag® Beads** by vortexing the bottle and add 2 ml of the resuspended **ClearMag® Beads** to 41 ml of **ClearMag® Binding Solution** in an appropriate vessel (user provided). Immediately transfer to a multi-channel reservoir.

Note

As time progresses the **ClearMag® Beads /ClearMag® Binding Solution** will slowly settle. Maintain the beads in suspension for uniform distribution to each well in the next step.

10. Mix the bead dispersion thoroughly and add 430 µl of the **ClearMag® Beads/ClearMag® Binding Solution** mixture to each well containing lysate. (20 µl of ClearMag® Beads + 410 µl of ClearMag® Binding Solution per well.)

11. Place the KingFisher® Microtiter Deep Well 96 Plate containing the lysate/**ClearMag®** mixture on the deck as indicated in the display on the instrument.

12. It requires three (3) KingFisher® Microtiter Deep Well 96 Plates for this step. For the first plate, place 900 µl of **ClearMag® Wash Solution** into each well. For the remaining two plates, place 900 µl of **100% ethanol** into each well. Place each plate on the deck as indicated in the display, with the **ClearMag®** wash plate coming before two ethanol wash plates.

13. Place 50-100 µl of the **ClearMag® RNase-Free Water** into each corresponding well of a KingFisher® 96 KF plate and place on the deck as indicated.

14. Initiate the KingFisher® MO BIO PowerMag® Blood DNA/RNA Isolation protocol program.

15. Upon completion, cover the wells of the KingFisher® 96 KF plate with an appropriate storage seal (user provided). DNA and RNA are now ready for downstream applications. No further steps are required.

We recommend storing eluents frozen at -80°C to maintain RNA integrity.

Thank you for choosing the PowerMag® Blood DNA/RNA Isolation Kit.

KingFisher® Duo Protocol (continued from step 7 on page 12)

See Consumables on page 7 for the list of necessary user-supplied materials.

8. Transfer the entire WBC lysate (from step 6, approx. 550 µl) to the twelve (12) wells of row (A) on a KingFisher® Microtiter Deep Well 96 Plate.

Note

The Duo limits the number of samples you can process at one time. If you are processing more than 12 samples you may place any additional sample lysates in the plates at 4°C until they are ready to be processed.

9. For each plate to be processed, resuspend the **ClearMag® Beads** by vortexing the bottle and add 280 µl of the resuspended **ClearMag® Beads** to 5.74 ml of **ClearMag® Binding Solution** in an appropriate vessel (user provided).

Note

As time progresses the **ClearMag® Beads /ClearMag® Binding Solution** will slowly settle. Maintain the beads in suspension for uniform distribution to each well in the next step.

10. Mix the bead dispersion thoroughly and add 430 µl of the **ClearMag® Beads/ClearMag® Binding Solution** mixture to each well containing lysate. (20 µl of ClearMag® Beads + 410 µl of ClearMag® Binding Solution per well.)

11. Place 900 µl of **ClearMag® Wash Solution** into each well of **row (C)**. Place 900 µl of **100% Ethanol** into each well of **rows (D & E)** and place plate on the KingFisher® Duo deck.

12. Place a KingFisher® Duo **12-tip comb** into the second **row (B)** of the KingFisher® Microtiter Deep Well 96 Plate.

13. Place 50-100 µl of the **ClearMag® RNase-Free Water** into each well of a KingFisher® Duo Elution Strip and place on the deck.

14. Initiate the KingFisher® Duo MO BIO PowerMag® Blood DNA/RNA Isolation Kit protocol program.

15. Upon completion, cover the wells of the KingFisher® Duo Elution Strip with an appropriate storage seal (user provided). DNA and RNA are now ready for downstream applications. No further steps are required.

We recommend storing eluents frozen at -80°C to maintain RNA integrity.

Thank you for choosing the PowerMag® Blood DNA/RNA Isolation Kit.

HINTS AND TROUBLESHOOTING GUIDE

Blood Preservation Methods that can be used with this kit

There is **NO** change in protocol when using preserved blood but your blood sample will be diluted with the addition of preservation solution.

- PAXgene™ Tubes (www.preanalytix.com)
- Biomatrica RNAgard® Blood System (www.biomatrica.com/rnagardblood_tube.php)

Use the manfacturer's protocol recommendations for preservation of whole blood.

If DNA/RNA does not PCR amplify

- Check DNA and RNA yields by gel electrophoresis and spectrophotometer readings. DNA template is typically added to 10 ng per reaction, although more or less may be needed depending on the reaction conditions, enzyme activity, and copy number of the target sequence.
- If DNA does not amplify after altering the amount of template in the reaction, PCR optimization (i.e. changing reaction conditions, validating primers, or testing a different polymerase) should be attempted.

Concentrating DNA and RNA

The final volume of eluted nucleic acids will be 50-100 µl. Nucleic acids may be concentrated by adding 5 µl of 5 M NaCl and inverting 3-5 times to mix. Next, add 220 - 250 µl of 100% cold ethanol and invert 3-5 times to mix. Incubate at -20°C for at least 10 minutes to overnight for DNA and overnight at -20°C for RNA (for optimal recovery). Centrifuge at 13,000 x g for 15 minutes. Decant all liquid. Wash the nucleic acid pellet with 70% cold ethanol. Centrifuge at 13,000 x g for 10 minutes to re-pellet the sample. Decant ethanol and dry in a speed vacuum, desiccator, or ambient air. Resuspend precipitated nucleic acids in sterile water or sterile 10 mM Tris.

Note

This procedure must be done individually after transferring the eluted sample to a microcentrifuge tube.

Storing DNA and RNA

The nucleic acids are eluted in ClearMag® RNase-Free Water. Store eluents at -80°C to prevent degradation. DNA and RNA can be eluted in 10 mM Tris buffer pH 7, or TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. Prolonged storage in the microplates at 4°C will result in the loss of liquid due to evaporation.

MO BIO offers TE (10 mM Tris, 0.1 mM EDTA, pH 8.0) which will allow for maximal protection of DNA and RNA during storage with no PCR inhibition (Catalog# 17320-1000).

PRODUCTS RECOMMENDED FOR YOU

Product	Catalog#	Amount	This is for you if…
UltraClean® Blood DNA Isolation Kit (Non-Spin)	12000-100	100 preps	You are interested in isolating high molecular weight DNA from whole blood without spin filters.
UltraClean® BloodSpin® DNA Isolation Kit	12200-50	50 preps	You need a fast spin column protocol for isolating DNA from 200 µl of whole blood.
	12200-250	250 preps	
BiOstic® Bacteremia DNA Isolation Kit	12240-50	50 preps	You need to isolate bacterial DNA from cultured blood.
DNase Max™ Kit	15200-50	1 ml	You plan to remove genomic DNA from a mixture of nucleic acids.
RNase A (25 mg/ml Solution)	1202-1	1 ml	You need to remove RNA from a mixture of nucleic acids.

TECHNICAL SUPPORT

Phone: Toll Free 800-606-6246, or 760-929-9911
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Committed to resolving your technical questions promptly, our technical support team is trained to work with you to rapidly and effectively trouble shoots any issues. We commit to providing you with relevant online support resources that help you complete your research projects.

Frequently Asked Questions:
www.mobio.com/faq

SDS:
www.mobio.com/sds

Protocols:
www.mobio.com/protocols

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Ordering Information:
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