MO BIO Laboratories, Inc.

Saving You Time For Life

PowerMag® DNA Clean-Up Kit

(Optimized for KingFisher®)

Catalog No. 27900-4-KF

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

INSTRUCTION MANUAL

Version 04302015

Please recycle

www.mobio.com | T: 800.606-6246 | T: 760.929-9911 | technical@mobio.com

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KIT CONTENTS

	Kit Catalog #22100-4-KF	
Component	**Catalog#**	**Amount**
PowerMag® IRT Solution 1	27900-4-KF-1	25 ml
PowerMag® IRT Solution 2	27900-4-KF-2	22 ml
ClearMag® Binding Solution	27900-4-KF-3	200 ml
ClearMag® Beads	27900-4-KF-4	9 ml
PowerMag® Elution Buffer	27900-4-KF-5	44 ml
96 Well V-Bottom Plates	27900-4-KF-VP	4 plates
Sealing Tape	27900-4-KF-ST	8 tapes

KIT STORAGE

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

PRECAUTIONS

Please wear gloves, laboratory coat and safety glasses when using this product. Avoid skin contact with kit reagents. In case of contact, wash the affected area thoroughly with soap and water. Do not ingest. See Safety Data Sheets (SDS) for emergency procedures in case of accidental contact or ingestion. SDS information is available upon request (760-929-9911) or at www.mobio.com/MSDS

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

REQUIRED BUT NOT INCLUDED

Equipment

- ☐ Centrifuge capable of handling two 96 Well Plates (13 cm x 8.5 cm x 1.4 cm) at 4500 x g
- ☐ Multichannel Pipettor (s) (volumes of 50 µl - 1000 µl)
- ☐ Single pipettor(s) (volumes of 10 µl – 1000 µl)
- ☐ Orbital plate shaker capable of 450 rpm

Optional

- Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)

Reagents & Consumables Required but not Included

- 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 website.
- Multi-channel pipettor reagent reservoirs
- 15 ml conical tube (for Duo protocol)
- 50 ml conical tube (for Flex protocol)
- 100% ethanol (Molecular Biology Grade)
- Water (Molecular Biology Grade)

PROTOCOL OVERVIEW

PowerMag®
DNA Clean-Up Kit

(Optimized for KingFisher®)
Catalog No. 27900-4-KF

The PowerMag® DNA Clean-Up Kit is optimized for use with the Thermo Scientific KingFisher Flex and KingFisher® Duo platforms.

The PowerMag® DNA Clean-Up Kit utilizes our patented Inhibitor Removal Technology® (IRT) and ClearMag® magnetic bead technology to provide researchers with a high-throughput, automated method for removing PCR inhibitory contaminants from up to 20 µg of previously isolated genomic DNA. Using the same DNA purification chemistry as our popular PowerClean® Pro DNA Clean-Up Kit, the PowerMag® DNA Clean-Up Kit streamlines your DNA purification workflow by enabling true hands-off, walk-away DNA purification.

The PowerMag® DNA Clean-Up Kit will remove sources of sample discoloration that prevent accurate DNA quantification by UV-VIS absorption and will remove PCR inhibiting substances, such as humic acids, heme, polysaccharides, polyphenols, fulvic acids, dyes and certain inhibitory ions. The resulting high purity DNA ensures a successful outcome to downstream applications, such as restriction digestion, PCR amplification and next generation sequencing. The PowerMag® DNA Clean-Up chemistry has been validated with DNA isolated from a variety of problematic sources including soil, water, plants, stool and biofilms.

Archived or previously isolated DNA samples are purified when combined with our proprietary DNA Clean-Up reagents. Inhibitors are selectively removed from the DNA solution via flocculation and the resulting DNA-containing supernatant is combined with ClearMag® Beads and a proprietary binding buffer in a KingFisher® deep-well plate. DNA is captured on the ClearMag® Beads, washed with ethanol and eluted with 10 mM Tris, pH 8.0 buffer. Purified DNA is ready for PCR analysis and other downstream applications.

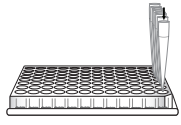
The PowerMag® DNA Clean-Up Kit is optimized for use with the Thermo Scientific KingFisher® robotic platforms but other open platform robots may be used with this kit. To adapt this kit to your specific automation platform, you may need to contact your local field application scientist of the manufacturer of your robot for help in adapting this protocol to your system.

Note

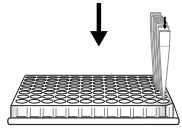
The order of placement of components and reagents for the platform portion of the protocol will be described in the downloaded software specific to your KingFisher® platform.

PowerMag® DNA Clean-Up Kit

Prepare Sample

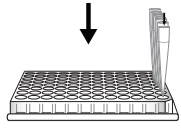


- Add up to 20 µg of dsDNA in a volume ≤ 100 µl

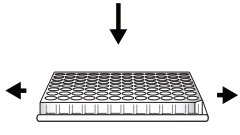


- Add Molecular Biology Grade H_2O to samples to bring the final volume of each well to 100 µl

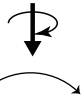
Inhibitor Removal Technology®



- Add PowerMag® IRT Solution 1
- Add PowerMag® IRT Solution 2

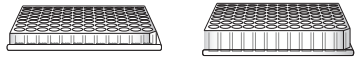


- Shake/Vortex briefly



- *Centrifuge*

DNA Isolation



Transfer the supernatant to a clean KingFisher® Deep Well Plate

PROTOCOL

Please wear gloves at all times.

Important Notes Before Starting:

- This kit was designed to work with up to 20 µg of input DNA and has been used successfully with as little as 20 ng. Using more than 20 µg will decrease the efficiency of inhibitor removal and can lead to loss of nucleic acid.
- For best results, your input DNA samples should be in water or Tris buffer, pH 8.0. We do not recommend using this kit with buffers containing > 1 mM EDTA.
- You will need 300 ml of 100% ethanol for each full 96 well plate being processed on the KingFisher® Flex. The KingFisher® Duo requires 40 ml of 100% ethanol for each 12 wells processed.

1. Add up to 100 µl of DNA sample to each well of a 96 Well V-Bottom Plate (provided). If less than 100 µl is added, adjust the sample volume to 100 µl with molecular biology-grade water (user provided).

2. Add 50 µl of PowerMag® IRT Solution 1 to each well containing DNA. Mix by repeated pipetting (3X).

Note

If processing a full 96-well plate, you may wish to dispense IRT Solution 1 and Solution 2 (next step) from a reagent reservoir using a multichannel pipettor or by using a repeating pipettor (e.g., Eppendorf's Repeater® M-4 with CombiTips) to reduce processing time.

3. Add 50 µl of PowerMag® IRT Solution 2 to each well containing DNA. Seal the plate with a Sealing Tape and mix the well contents by gently tapping on the sides of the sealed plate.

Note

The IRT reaction is instantaneous and, as such, does not require a lot of mixing. Shaking the sealed plate gently side-to-side in your hand is sufficient.

4. Centrifuge the plate at 4,500 x g for 6 minutes at room temperature with an appropriately weighed balance plate.

Note

During centrifugation the platform-specific volumes of ClearMag® Bead/Bind and ethanol can be prepared and loaded into KingFisher® deep well plates.

5. To run the DNA purification on the KingFisher® Flex, go to step 6 page 12 and for the KingFisher® Duo, go to page 14.

KingFisher® Flex Protocol (continued from step 5)

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

6. For each 96 well plate to be processed on the KingFisher® Flex, prepare a 50 ml conical tube (user provided) containing 45 ml of ClearMag® Binding Solution and 2 ml of ClearMag® Beads. Mix well to obtain a homogeneous dispersion of beads.

Note

As time progresses the ClearMag® Bead/Binding Solution will slowly settle. Maintain the beads in suspension for uniform distribution to each well in the next step by vortexing the dispersion periodically.

7. Add 470 µl of the ClearMag® Bead/Binding Solution to each well of a KingFisher® Microtiter Deep Well 96-well plate.

8. Remove and discard the Sealing Tape from the plate centrifuged in Step 4. A visually discernible pellet will be present at the bottom of each well. Avoiding this pellet, transfer the entire volume (expect 150 – 190 µl) of supernatant from each well in to its respective well in the KingFisher® Microtiter Deep Well Plate prepared in Step 7. We recommend that you use an 8-tip or 12-tip multichannel pipettor at this step to reduce total processing time. You may find it helpful to place the 96 well plate on top of a colored (i.e. non-white) support to aid in visualizing the IRT pellet at the bottom of each well.

Note

To avoid disrupting the IRT pellet during removal of the supernatant, we recommend that you insert your pipet tips along the front wall of each well during the aspiration step. By placing your pipet tips in physical contact with the well wall and sliding the tips to the bottom of the wall, the tips will avoid contact with the IRT pellet. Aspirating fluid from the center of the well is not recommended.

9. Open the KingFisher® Flex-specific PowerMag® DNA Clean-Up program on your instrument.

10. Place the KingFisher® Microtiter Deep Well 96 Plate containing the IRT-treated DNA samples and ClearMag® Bead/Binding Solution onto the robotic deck at the specified location as indicated in the PowerMag® DNA Clean-Up program.

11. Place 1 ml of 100% ethanol (user provided) into each well of three clean KingFisher® Microtiter Deep Well 96 plates and place on the deck at the specified locations as indicated in the program.

12. Place 50 – 100 µl of PowerMag® Elution Buffer into each well of a KingFisher® 96 KF Elution plate and place on the deck at the specified location. Initiate the KingFisher® MO BIO PowerMag® DNA Clean-Up robotic program.

13. Upon completion of the robotic program, cover the wells of the KingFisher® 96 KF Elution plate with an appropriate storage seal (user provided). DNA is now ready for any downstream applications.

We recommend storing eluted DNA at -20 to - 80°C for long term stability.

Thank you for choosing the PowerMag® DNA Clean-Up Kit.

KingFisher® Duo Protocol (continued from step 5 on page 12)

6. For each group of 12 samples to be processed on the KingFisher® Duo, prepare a 15 ml conical tube (user provided) containing 5.625 ml of ClearMag® Binding Solution and 250 µl of ClearMag® Beads. Mix well to obtain a homogeneous dispersion of beads.

Note

As time progresses the ClearMag® Bead/Binding Solution will slowly settle. Maintain the beads in suspension for uniform distribution to each well in the next step by vortexing the dispersion periodically.

7. Add 470 µl of the ClearMag® Bead/Binding Solution to each well in Row A of a KingFisher® Microtiter Deep Well 96-well plate.

8. Remove and discard the Sealing Tape from the plate centrifuged in Step 4. A visually discernible pellet will be present at the bottom of each well. Avoiding the pellet, transfer the entire volume (expect 150 – 190 µl) of supernatant from each well in to its respective well in the KingFisher® Microtiter Deep Well Plate prepared in Step 7. We recommend that you use an 8-tip or 12-tip multichannel pipettor at this step to reduce total processing time. You may find it helpful to place the 96 well plate on top of a colored (i.e. non-white) support to aid in visualizing the IRT pellet at the bottom of each well.

Note

To avoid disrupting the IRT pellet during removal of the supernatant, we recommend that you insert your pipet tips along the wall of each well during the aspiration step. By placing your pipet tips in physical contact with the well wall and sliding the tips to the bottom of the wall, the tips will avoid contact with the IRT pellet. Aspirating fluid from the center of the well is not recommended.

9. Place a KingFisher® Duo 12-tip comb into the second Row B of the KingFisher® Microtiter Deep Well 96 Plate.

10. Place 1 ml of 100% ethanol into each well of Rows C, D & E of the KingFisher® Microtiter Deep Well 96 Plate and put the plate on the instrument deck.

11. Place 50 - 100 µl of PowerMag® Elution Buffer into each well of a KingFisher® Duo Elution Strip and place on the deck at the specified location. Initiate the KingFisher® MO BIO PowerMag® DNA Clean-Up robotic program.

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

We recommend storing eluted DNA at -20 to - 80°C for long term stability.

Thank you for choosing the PowerMag® DNA Clean-Up Kit.

HINTS AND TROUBLESHOOTING GUIDE

Centrifuge with a Maximum Speed Less Than 4500 x g

Multiply the protocol time and speed to determine the total force (or speed) required (x g). Divide the total by the maximum speed of your centrifuge (round up if necessary). This will be the number of minutes your centrifuge will need to run to achieve the appropriate overall force.

Example: 10 minutes at 4500 x g = 45000.
If your centrifuge has a maximum speed of 2500 x g, divide 45000 ÷ 2500 = 18 minutes of centrifugation.

Amount of DNA to Process

This kit is designed to process up to 100 µl of DNA (20 µg maximum). For inquiries regarding the use of larger sample amounts, please contact technical support for suggestions.

Quantifying Isolated DNA: UV-VIS spectrophotometry vs. Qubit® (or Picogreen)

Due to the fact that UV-VIS spectrophotometric analysis of nucleic acids (e.g., NanoDrop®) will report the total absorbance profile of a sample (i.e., the contributions of DNA/RNA and any non-nucleic acid, UV-absorbing compounds present in the sample), this method is susceptible to reporting DNA concentrations as being higher than they actually are. For this reason, we recommend that customers use UV-VIS spectrophotometry to assess sample quality (260/280, 260/230 ratios) and a nucleic acid specific assay, such as the Qubit® assay, for accurate DNA quantification in place of UV-VIS analysis.

Note

We have determined that a small amount of ClearMag® Binding Buffer will be present in eluted DNA. The residual Bind will not negatively impact PCR, NGS library preparation or any other downstream process but will inflate your DNA concentration measurements made by UV-VIS/NanoDrop®. If you are using only UV-Vis spectrophotometry to quantify your isolated DNA, we recommend that you run a 'blank' DNA isolation alongside your samples when running this kit. An appropriate blank is 450 µl ClearMag® Bind, 20 µl ClearMag® Beads and 200 µl PCR-grade water. Run this blank alongside your samples using the same ethanol wash and elution conditions; subtract the ng/µl value you obtain for your blank via NanoDrop® (typically 10 ng/µl) from your measured samples to obtain more accurate quantification.

If DNA Does Not Amplify

Make sure to check DNA integrity by gel electrophoresis and concentration by an appropriate method of quantification (see above). Template DNA concentration could influence the outcome of PCR along with other the reaction conditions, enzyme activity, and copy number of the target sequence. If DNA does not amplify after

altering the concentration of template DNA, please call our technical support for suggestions.

Eluted DNA Sample Is Brown
We have not observed any coloration in DNA isolated using the PowerMag® DNA Clean-Up Kit. If you observe coloration in your samples, please contact technical support for suggestions.

Concentrating the DNA
If your sample requires further concentration, add a volume of 5 M NaCl equal to 1/10th the volume of your eluent and invert 3-5 times to mix. Next, add 2.5 volumes of 100% cold ethanol and mix. Incubate at -20°C for 20 minutes. Centrifuge at 16,000 x g for 20 minutes at room temperature. Decant all liquid. Remove residual ethanol in a vacuum concentrator (such as a SpeedVac™), a desiccator, or ambient air. Resuspend precipitated DNA in sterile water or 10 mM Tris.

Storing DNA
DNA is eluted in PowerMag® Elution Buffer (10 mM Tris) and must be stored at -20°C to -80°C to prevent degradation. DNA can be eluted in TE but the EDTA may inhibit reactions such as PCR and automated sequencing. DNA may also be eluted with sterile DNA-Free PCR Grade Water (MO BIO Catalog# 17000-10).

Technical Tips
Visit MO BIO's The Culture Dish at http://www.mobio.com/blog/ for the latest in technical tips for frequently asked questions. Use this valuable resource to share your suggestions and optimization techniques for difficult or problematic samples.

PRODUCTS RECOMMENDED FOR YOU

Product	Catalog#	Amount	This is for you if...
PowerClean® Pro DNA Clean-Up Kit	12997-50	50 preps	You need a manual kit for secondary clean-up of DNA in just 7 minutes.
PowerClean® Pro RNA Clean-Up Kit	13997-50	50 preps	You need a manual kit for secondary clean-up of RNA in just 7 minutes.
PowerMag® Microbiome RNA/DNA Isolation Kit (Optimized for KingFisher®)	27600-4-KF	4 x 96 preps	You need a high-throughput, hands-free kit for isolation of nucleic acids from stool and gut samples.
PowerMag® Soil DNA Isolation Kit Sample (Optimized for KingFisher®)	27000-4-KF	4 x 96 preps	You are looking for a high-throughput, hands-free method of isolating DNA from soil samples.

TECHNICAL SUPPORT

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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.

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