PowerMag®
Microbial DNA Isolation Kit

Catalog No.	Quantity	Total Purifications
27200-4	4 x 96 Preps	384

Instruction Manual

Please recycle

Version: 06282016

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Introduction

The PowerMag® Microbial DNA Isolation Kit is optimized for use with the Thermo Scientific KingFisher® Flex, the KingFisher® Duo and the Eppendorf epMotion® 5075 TMX platforms.

The PowerMag® Microbial DNA Isolation Kit can be used for automated isolation of high quality genomic DNA from pure microbial cultures, food cultures and swabs. A variety of microorganisms including bacterial spores, fungal types, and food samples such as meats, cheeses and dairy products, chocolate, fruits, vegetables, and juices have been tested successfully with this kit. The protocol is designed for isolation of up to 450 µl of lysate in Deep Well Plates. The PowerMag® Microbial DNA Isolation Kit includes a patented Inhibitor Removal Technology® (IRT) step to remove PCR-inhibiting compounds associated with food cultures, including lipids and polysaccharides. A novel, proprietary magnetic bead system is used for the isolation of nucleic acids, resulting in inhibitor-free DNA that is ready to use in the most demanding downstream applications including PCR, qPCR and next generation sequencing.

This kit requires the use of a specialized plate shaker for 96 well homogenization of samples in blocks. We recommend the Retsch 96 Well Plate Shaker (MO BIO Catalog# 11996 in the USA only. For information outside the USA, contact technical@mobio.com) and Adapters (MO BIO Catalog# 11990). For low through-put platforms such as the KingFisher® Duo, homogenization may also be performed in 2 ml bead tubes using a Vortex Genie® 2 or a high powered bead beater such as the PowerLyzer® 24. With both of these methods, lysates need to be transferred individually to the appropriate Deep Well 96 Plates for automation on the KingFisher® or epMotion®.

> **Note**: The order and placement of components and reagents for the platform portion of the protocol will be described in the downloaded software specific to your platform.

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

Protocol Overview

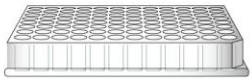
Pelleted cells are resuspended in the provided lysis solution containing RNase A, added to a 96 well bead beating plate and subjected to rapid and thorough homogenization. Cell lysis occurs by a combination of mechanical and chemical methods. Our patented Inhibitor Removal Technology® is included as part of the protocol providing efficient contaminant removal and improved DNA yield. Total genomic DNA is captured on magnetic beads in the presence of ethanol which eliminates the use of any chaotropic salts. DNA on the beads is washed and then eluted using a low salt buffer (10 mM Tris pH 8). The eluted DNA is ready for qPCR, next generation sequencing, and other downstream applications.

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

Other Related Products	Catalog No.	Quantity
96 Well Plate Shaker	11996	1 unit (120 V)
QIAGEN TissueLyser II (see www.QIAGEN.com for details)	85300	1 unit (100–120/220–240 V)
PowerMag® Magnetic Separator	27400	1 unit
Plate Adapter Set	11990	1 set

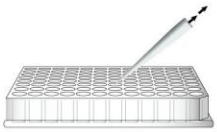
PowerMag® Microbial DNA Isolation Kit

Collect Cells



- Add liquid culture to PowerMag® 2 ml Collection Plate

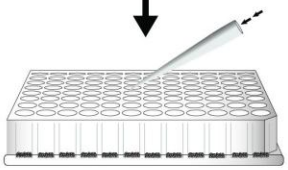
- *Centrifuge*



- Remove supernatant
- Add PowerMag® MicroBead Lysis Solution/RNase A

- *Vortex to resuspend cell pellet*

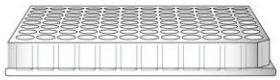
Cell Lysis



- Transfer resuspended cells into PowerMag® Bead Plate
- Place on the 96 Well Plate Shaker and Shake

- *Centrifuge*

Inhibitor Removal Technology®



- Transfer the supernatant to a clean PowerMag® 1 ml Collection Plate
- Add IRT Solution
- Incubate 4°C

- *Centrifuge*

DNA ISOLATION
Follow KingFisher® protocol

DNA ISOLATION
Follow epMotion® protocol

Equipment Required

- Centrifuge capable of handling two 96 Well Blocks (13 cm x 8.5 cm x 6.0 cm) at 4500 x *g*
  Note: If you have a centrifuge with a maximum speed less than 4500 x *g* see the Hints and Troubleshooting Guide.
- Multi-channel Pipettor(s) (volumes of 50 µl - 1000 µl)
  Note: The KingFisher® Duo applications require a 12 channel pipettor if multichannel pipetting is desired on that platform.
- Single Pipettor(s) (volumes of 5 µl – 500 µl)
- Mechanical Shaker for 96 Well Blocks (MO BIO Catalog# 11996 or QIAGEN Catalog# 85300) and Plate Adapters (MO BIO Catalog# 11990)
- Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)
- Optional: Vortex Adapter (MO BIO Catalog# 13000-V1-24)

Reagents and Consumables Required but not Included

- Contact your Thermo Scientific representative for the KingFisher® plastic disposables or your Eppendorf representative for the epMotion® plastic disposables specific to your platform.
  Note: A list of consumables that are required but not provided can be found at www.mobio.com/powermag.
- Eppendorf epMotion® users require the following plastics (not provided):
  - 2 ml Deep Well Plates (DWP): Greiner, 96W MASTERBLOCK, PPN, 2ml, CLEAR cat # 780280 (4 plates required)
  - Microplates (MO BIO MTP): Greiner, 96 Well Plate, PP, Round (U) Bottom, Chimney Style, Natural (clear) cat # 650201 (8 plates required)
  - Elution Sealing Mats: Greiner, Cap Mats, Fits 1.2ml MASTERBLOCK, EVA (ETHYLENE) cat # 381070 (4 mats required)

  Note: The PowerMag® epMotion® Accessory Pack (MO BIO Catalog# 27300-4-EP) is no longer available.
- Appropriate pipet tips for the Multi-channel pipettors to be used in the lysate preparation steps.
  Note: The tips must fit in the round wells of the 1 ml blocks (examples of these are Molecular Bioproducts ART Catalog# 2179-HR, Eppendorf Catalog# 0030 077.750 and Rainin Catalog# RT-1000F).
- KingFisher® users need reagent reservoirs for 5 – 300 ml volumes.
- KingFisher® Duo users will need appropriate 96 well storage plates/seals capable of holding 450 µl.
- 100% Ethanol is required for all protocols.

Kit Contents

	Kit Catalog# 27200-4	
Component	**Catalog #**	**Amount**
PowerMag® Bead Plates	27200-4-BP	4
PowerMag® MicroBead Lysis Solution	27200-4-1	150 ml
PowerMag® IRT Solution	27200-4-2	44 ml
SwiftMag® Beads	27200-4-3	22 ml
SwiftMag® Elution Buffer	27200-4-4	49 ml
RNase A Solution (25 mg/ml)	27200-4-5	1.5 ml
PowerMag® 1 ml Collection Plates	27200-4-1CP	4
PowerMag® 2 ml Collection Plates	27200-4-2CP	4
Sealing Tape	27200-4-ST	32
Round Well Mats	27200-4-RWM	4

Kit Storage
RNase A Solution should be stored at 4°C.
The other 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 Material Safety Data Sheets for emergency procedures in the event of accidental ingestion or contact. All MSDS 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.

Protocol

Please wear gloves at all times

Important Notes Before Starting:

- Warm the PowerMag® MicroBead Lysis Solution at 60°C for 10 minutes before use. Use the PowerMag® MicroBead Lysis Solution while still warm.
- Before starting, add 9 µl of the provided RNase A Solution per 1 ml of warmed PowerMag® MicroBead Lysis Solution. Each 96 well plate will require exactly 33.6 ml of this mixture. To allow for pipetting variations and overage for the reagent reservoir, it is suggested to add 315 µl of the RNase A Solution to 35 ml of the PowerMag® MicroBead Lysis Solution. For KingFisher® Duo applications add 350 µl of the warmed PowerMag® MicroBead Lysis Solution to each well followed by 3 µl of the RNase A Solution to each.
- You will need 333 ml of 100% Ethanol for each full 96 well plate being processed on the KingFisher® and 363 ml for the epMotion®. The KingFisher® Duo requires 36 ml for each 12 wells processed.
- Before first use, centrifuge the bead plates for 3 minutes at 4500 x *g* before removing and discarding the mat.

1. Dispense 1.8 ml of liquid culture into each well of the PowerMag® 2 ml Collection Plate, and cover with Sealing Tape. Centrifuge at 4500 x *g* for 12 minutes.

2. Discard tape and remove the media without disturbing the cell pellet. It is okay to leave a small amount of residual media in each well.
   Note: Excessive residual media will begin to dilute the lysis chemistry.

3. Add **350 µl of PowerMag® MicroBead Lysis Solution/RNase A Solution** and apply a new piece of Sealing Tape (provided). Resuspend the cell pellet completely in the PowerMag® MicroBead Solution by high speed vortexing. Centrifuge very briefly to ensure that all of the cell suspension is at the bottom of the wells.
   Note: Avoid repelleting the cells.

4. Remove the **Round Well Mat (after centrifuging for 3 minutes at 4500 x *g*)** from the **PowerMag® Bead Plate** and discard. Transfer the resuspended cells into the **PowerMag® Bead Plate**.

5. Seal the **PowerMag® Bead Plate** with a new **Round Well Mat** (provided).

6. Place each of the **PowerMag® Bead Plates** (with **Round Well Mats** securely affixed) between 2 adapter plates (MO BIO Catalog# 11990) and place on the 96 Well Plate Shaker (MO BIO Catalog# 11996). Reference the protocol provided with the adapter plates for proper placement. Shake at speed 20 for 5 minutes.

7. Remove plates and re-orient them so that the side closest to the machine body is now furthest from the machine body and shake again at speed 20 for 5 minutes.

8. Centrifuge the **PowerMag® Bead Plates** for 6 minutes at 4500 x *g.*

9. Remove and discard **Round Well Mat**. Avoiding the glass beads as best as possible, transfer the supernatant to a clean **PowerMag® 1 ml Collection Plate**.
   Note: Supernatant may still contain some beads.

10. Add **100 µl of PowerMag® IRT Solution** to the wells of the plate and cover with Sealing Tape. Vortex for 5 seconds. Incubate at 4°C for 10 minutes.
    Note: This step removes any inhibitors that may be present and also any glass beads that may have carried over.

11. Centrifuge the plate for 9 minutes at 4500 x *g*.

12. Open the appropriate protocol on your instrument specific to your platform and then proceed. For KingFisher® Flex applications go to page 8, for epMotion® go to page 9 and for KingFisher® Duo go to page 10.

KingFisher® Flex Protocol (continued from step 12)

13. Avoiding the pellet, transfer up to 450 µl of supernatant to the appropriate wells on a KingFisher® Microtiter Deep Well 96 Plate.

    Note: You may place the supernatant in the plate at 4°C for several hours if you need to stop during the protocol or if you can only process one 96 well plate at a time.

14. For each plate to be processed, resuspend the **SwiftMag® Beads** by vortexing the bottle and add 5 ml of the resuspended **SwiftMag® Beads** to 45 ml of **100% Ethanol in an appropriate vessel (user provided). Immediately transfer to a multi-channel reservoir.**

    Note: As time progresses the **SwiftMag® Beads /100% Ethanol** will slowly settle. Maintain the beads in suspension for uniform distribution to each well in the next step.

15. Mix well and add 500 µl of the **SwiftMag® Beads /100% Ethanol** to each well containing lysate.

16. Place the KingFisher® Microtiter Deep Well 96 Plate containing the lysate/Magnetic beads and Ethanol on the deck as indicated in the display on the instrument.

17. It will require three (3) KingFisher® Microtiter Deep Well 96 Plates for the next step. Place 1 ml of **100% Ethanol** into each corresponding well of three (3) KingFisher® Microtiter Deep Well 96 Plates and place on the deck as indicated in the display.

18. Place 100 µl of the **SwiftMag® Elution Buffer** into each corresponding well of a KingFisher® 96 KF plate and place on the deck as indicated.

19. Initiate the KingFisher® MO BIO PowerMag® Microbial DNA Isolation protocol program.

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

    We recommend storing DNA frozen (-20°C or -80°C). SwiftMag® Elution Buffer is 10 mM Tris pH 8.0 and does not contain EDTA.

Thank you for choosing the PowerMag® Microbial DNA Isolation Kit.

epMotion® Protocol (continued from step 12)

13. Avoiding the pellet, transfer up to 450 µl of supernatant to the appropriate wells on a Greiner 2 ml Deep Well Plate (DWP) (see page 5 for details on Greiner 2 ml Deep Well Plates) and place on the appropriate location on the deck. This location is specified when you download the MO BIO PowerMag® Microbial DNA Isolation program.
    Note: You may place the supernatant in the plate at 4°C for several hours if you need to stop during the protocol or if you can only process one 96 well plate at a time.

14. For each single plate to be processed, place 2 of the Greiner Microplates (MO BIO MTP) (see page 5 for details on Greiner Microplates) at the appropriate locations on the deck as indicated in the epMotion® software. These identical plates will be referred to as MO BIO MTP1 and MTP2 by the software.

15. For each single plate to be processed, place 318 ml of **100% Ethanol** into an Eppendorf 400 ml reservoir placed at the appropriate location on the deck as indicated in the epMotion® software.

16. For each single plate to be processed, place 11 ml of **SwiftMag® Elution Buffer** into an Eppendorf 30 ml reservoir placed in an Eppendorf tub holder located at the appropriate location on the deck as indicated.

17. For each single plate to be processed, resuspend the **SwiftMag® Beads** by vortexing the bottle and add 5 ml of the resuspended **SwiftMag® Beads** to 45 ml of **100% Ethanol in an appropriate vessel (user provided).** Mix well and place the entire volume into an Eppendorf 100 ml reservoir placed in an Eppendorf tub holder located at the appropriate location on the deck as indicated.

18. Initiate the epMotion® MO BIO PowerMag® Microbial DNA Isolation program.
    Note: It is imperative to start the protocol immediately otherwise the beads will begin to settle.

19. Upon completion, cover the wells of the Greiner Microplate (MO BIO MTP 2) with a Greiner Elution Sealing Mat (see page 5 for details on Greiner elution sealing mats) . DNA is now ready for any downstream application. No further steps are required.

    We recommend storing DNA frozen (-20°C or -80°C). SwiftMag® Elution Buffer is 10 mM Tris pH 8.0 and does not contain EDTA.

Thank you for choosing the PowerMag® Microbial DNA Isolation Kit.

KingFisher® Duo Protocol (continued from step 12)

13. Avoiding the pellet, transfer up to 450 µl of supernatant to the corresponding wells on an appropriate 96 well Plate (user provided).
    Note: Since the Duo limits the number of samples you can process at one time, you may place the remaining supernatant in the plates at 4°C for several hours.

14. Transfer lysate from up to twelve (12) wells to the first long row (A) on a KingFisher® Microtiter Deep Well 96 Plate.

15. Add 450 µl of **100% Ethanol** to each well in row A containing lysate.

16. Prepare the **SwiftMag® Beads** by vortexing the bottle to resuspend. Immediately add 50 µl of the resuspended **SwiftMag® Beads** to each well in row A containing the lysate/Ethanol mixture.
    Note: The beads will slowly settle so it is critical to make sure the beads stay in suspension.

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

18. Place 1 ml of **100% Ethanol** into each well of the next three (3) rows (C, D & E) on the plate and place on the deck.

19. Place 100 µl of the **SwiftMag® Elution Buffer** into each well of a KingFisher® Duo Elution Strip and place on the deck.

20. Initiate the KingFisher® Duo MO BIO PowerMag® Microbial DNA Isolation protocol program.

21. Upon completion, cover the wells of the KingFisher® Duo Elution Strip with an appropriate storage seal. DNA is now ready for any downstream application. No further steps are required.

    We recommend storing DNA frozen (-20°C or -80°C). SwiftMag® Elution Buffer is 10 mM Tris pH 8.0 and does not contain EDTA.

Thank you for choosing the PowerMag® Microbial DNA Isolation Kit.

Hints and Troubleshooting Guide

Difficult to Lyse Cells
When working with organisms that have proven to be difficult to lyse using mechanical or chemical methods, a 10 minute incubation at 70°C in the lysis buffer can be performed. Continue by proceeding with the mechanical lysis step using the 96 Well Plate Shaker.

Alternative Method for Enhancing Lysis using Freeze/Thaw Cycles
Add the samples to the PowerMag® Bead Plate and maintain at either -70°C or at -20°C until the samples are completely frozen. Immediately float the PowerMag® Bead Plate in a 65°C water bath. Repeat the freeze-thaw a second time.

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.

DNA Quantification
PicoGreen® measurement of DNA yields will be more accurate compared to spectrophotometry, which detects the presence of low molecular weight digested RNA that may be recovered during purification. We always recommend running an agarose gel (0.8-1.2%) of the final DNA to visually compare with the yield analysis.

If DNA does not PCR amplify
- Check DNA yield by gel electrophoresis and PicoGreen® or spectrophotometry. Template is typically added to 10-100 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.

Eluted DNA Sample has Color
If you are working with a sample that has high secondary byproduct content (for example, red pepper, chocolate, coffee), there is the unlikely possibility that your eluted solution may contain some color. If this occurs, try increasing the IRT Solution to 150 µl. If you still encounter coloring, please contact MO BIO Laboratories, Inc. for technical assistance.

DNA Floats Out of Well When Loaded on a Gel
This usually occurs because some residual 100% ethanol remains in the final sample. The beads need to be completely dried before elution to prevent ethanol carryover. Ethanol precipitation (described in "Concentrating the DNA") is the best way to remove residual wash solution.

Hints and Troubleshooting Guide cont.

Concentrating the DNA

The final volume of eluted DNA will be 100 µl. The DNA may be concentrated by adding 5 µl of 5M NaCl and inverting 3-5 times to mix. Next, add 200 µl of 100% cold ethanol and invert 3-5 times to mix. Incubate at -20°C for at least 10 minutes to overnight. Centrifuge at 13,000 x *g* for 15 minutes. Decant all liquid. Wash the DNA 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, dessicator, or ambient air. Resuspend the precipitated DNA in sterile water or sterile 10 mM Tris.

> **Note**: This procedure must be done individually for each sample after transferring the eluted sample to a microcentrifuge tube.

Alternatively, you can warm the samples to 65°C in the Greiner Microplate (MO BIO MTP), uncovered, to evaporate traces of ethanol from the samples in the plate.

Storing DNA

DNA is eluted in SwiftMag® Elution Buffer (10 mM Tris, pH 8). Store the DNA at -20°C to prevent degradation. DNA can be eluted in TE without DNA loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA may also be eluted with sterile DNA-Free PCR Grade Water (MO BIO Catalog# 17000-10). DNA that has been eluted into sterile water should be stored at -70°C. Prolonged storage in the Greiner Microplates (MO BIO MTP) at 4°C will result in the loss of liquid due to evaporation.

MO BIO offers TE-4 (10 mM Tris, 0.1 mM EDTA pH 8.0) which will not inhibit PCR while still providing maximal protection of DNA during storage (Catalog# 17320-1000).

Contact Information

Technical Support:
Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: technical@mobio.com
Fax: 760-929-0109
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Fax: 760-929-0109
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For the distributor nearest you, visit our website at www.mobio.com/distributors

Products recommended for you

For a complete list of products available from MO BIO Laboratories, Inc., visit www.mobio.com.

Description	Catalog No.	Quantity
Plate Adapter Set	11990	1 set
PowerFood® Microbial DNA Isolation Kit	21000-100	100 preps
PowerLyzer® UltraClean® Microbial DNA Isolation Kit	12255-50	50 preps
UltraClean® Microbial DNA Isolation Kit	12224-50 12224-250	50 preps 250 preps
UltraClean® -htp 96 Well Microbial DNA Kit	10196-4	4 x 96 preps
PowerLyzer® 24 Bench Top Bead-Based Homogenizer	13155	1 unit