OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® E-Z Pure

M1380-00	5 mL
M1380-01	50 mL
M1380-02	500 mL

January 2013

For research use only.Not intended for diagnostic testing.

Mag-Bind® E-Z Pure

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Manual Revision: January 2013

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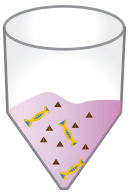
Introduction and Principle

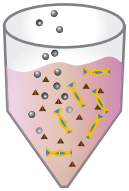
Omega Bio-tek's Mag-Bind® E-Z Pure Kit allows rapid and reliable isolation of PCR* products with high recovery rates. The system combines Omega Bio-tek's proprietary chemistries with the reversible nucleic acid-binding properties of magnetic beads that selectively binds PCR amplicons 100 bp and larger and eliminate excess nucleotides, primers, and small, non-targeted amplification products, such as primer dimers. This kit is designed for both manual and fully automated purification of PCR samples. Purified PCR fragments can be used for microarrays, automated fluorescent DNA sequencing, restriction enzyme digestion, and other applications.

The Mag-Bind® E-Z Pure magnetic particles technology provides a better solution for nucleic acid purification compared to centrifugation and vacuum-based technologies. The product can be easily scaled up while providing very user-friendly handling procedures. If using Mag-Bind® E-Z Pure for the first time, please read this booklet to become familiar with the procedures. PCR products are first mixed with Mag-Bind® E-Z Pure. PCR products then selectively bind to the Mag-Bind® E-Z Pure particles. With two rapid wash steps, trace contaminants such as nucleotides, primers and small, non-targeted amplification products are removed. Pure DNA is eluted in Elution Buffer or water. Purified DNA can be directly used in downstream applications without the need for further purification.

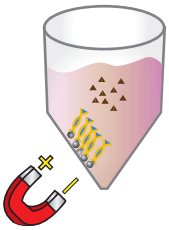
Illustrated Protocol



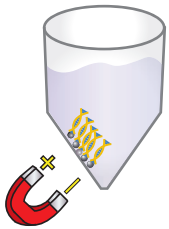
Measure the PCR Reaction



Add Mag-Bind® E-Z Pure and Mix

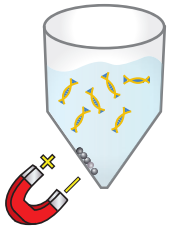


Magnetize and Remove Supernatant



Wash Twice with 70% Ethanol

Dry



Elute DNA

Kit Contents

Product Number	M1380-00	M1380-01	M1380-02
Mag-Bind® E-Z Pure	5 mL	50 mL	500 mL
User Manual	✓	✓	✓

Preparations

PCR Reaction Volume 96 well format	M1380-00 5 mL	M1380-01 50 mL	M1380-02 500 mL
10 µL	277 preps	2,777 preps	27,777 preps
25 µL	111 preps	1,111 preps	11,111 preps
50 µL	55 preps	555 preps	5,555 preps
100 µL	27 preps	277 preps	2,777 preps
PCR Reaction Volume 384 well format	**M1380-00 5 mL**	**M1380-01 50 mL**	**M1380-02 500 mL**
5 µL	555 preps	5,555 preps	55,555 preps
10 µL	277 preps	2,777 preps	27,777 preps
14 µL	198 preps	1,984 preps	19,841 preps

Storage and Stability

Mag-Bind® E-Z Pure is guaranteed for at least 12 months from the date of purchase when stored at 2-8°C.

Mag-Bind® E-Z Pure - 96-well Plate Protocol

Materials and Equipment to be Supplied by User:

- 96-well PCR plate containing PCR samples (up to 100 µL/well)
- Magnetic Separation Device (Recommended Cat# MSD-01)
- Vortexer
- Multichannel pipettor
- Reservoirs
- Sealing film
- 96-well microplate for elution
- 70% ethanol
- Elution Buffer (Cat# PDR048 or 10 mM Tris pH 8.5, TE Buffer, 0.1 mM EDTA, or diH_2O)
- 96-well processing plate (Note: the type of collection plate to be used depends on the type of magnetic separation stand used. For OBI's MSD-01, a 500 µL conical bottom microplate is recommended (Cat #EZ9604-02)).
- Optional: Oven capable of 37°C

1. Read the manufacturer's instruction manual for the magnetic separation device, if provided.

2. Place the 96-well PCR plate on the bench and measure the volume of the PCR reaction. Determine if transferring the sample to a processing plate is required. If necessary, transfer the PCR reactions to a 96-well microplate.

   Note: PCR Reactions >20 µL will need to be transferred to a processing plate. MSD-01 is not compatible with PCR plates. If processing in a PCR plate, a magnet compatible with PCR plates must be used. (Recommended V&P Scientific # VP 771H)

3. Shake the Mag-Bind® E-Z Pure to resuspend any Mag-Bind® E-Z Pure particles that may have settled. Add 1.8 volumes Mag-Bind® E-Z Pure to each well.

PCR Reaction Volume (µL)	Mag-Bind® E-Z Pure (µL)
10	18
25	45
50	90
100	180

4. Pipet up and down 5-10 times or vortex for 30 seconds.

5. Let sit at room temperature for 1 minute.

6. Place the plate on a magnetic separation device to magnetize the Mag-Bind® E-Z Pure. Let sit at room temperature until the Mag-Bind® E-Z Pure is completely cleared from solution.

7. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® E-Z Pure.

8. Add 200 µL 70% ethanol to each well.

9. Let sit at room temperature for 1 minute. It is not necessary to resuspend the Mag-Bind® E-Z Pure.

10. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® E-Z Pure.

11. Repeat Steps 8-10 for a second 70% ethanol wash step.

12. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the Mag-Bind® E-Z Pure. Remove any residue liquid with a pipettor.

    Note: It is important to dry the Mag-Bind® E-Z Pure before elution. Residual ethanol may interfere with downstream applications.

    Optional: Incubating the plate at 37°C can speed up the evaporation.

13. Remove the plate from magnetic separation device.

14. Add 30-40 µL Elution Buffer (or 10 mM Tris pH 8.5, TE Buffer, 0.1 mM EDTA, or diH_2O) to each well.

15. Pipet up and down 20 times or vortex for 30 seconds.

16. Let sit at room temperature for 2-3 minutes.

17. Place the plate on a magnetic separation device to magnetize the Mag-Bind® E-Z Pure. Let sit at room temperature until the Mag-Bind® E-Z Pure is completely cleared from solution.

18. Transfer the cleared supernatant containing purified DNA to a new 96-well microplate and seal with non-permeable sealing film.

19. Store the plate at 2-8°C if storage is only for a few days. For long-term storage, samples should be kept at -20°C.

Mag-Bind® E-Z Pure - 384-well Plate Protocol

Materials and Equipment to be Supplied by User:

- 384-well PCR plate containing PCR samples (up to 100 µL/well)
- Magnetic separation device for 384-well PCR plates
- Vortexer
- Multichannel pipettor
- Reservoirs
- Sealing film
- 70% ethanol
- Elution Buffer (Cat# PDR048 or 10 mM Tris pH 8.5, TE Buffer, 0.1 mM EDTA, or diH_2O)
- Skirted 384-well PCR plate
- Optional: Oven capable of 37°C

1. Read the manufacturer's instruction manual for the magnetic separation device, if provided.

2. Place the 384-well PCR plate on the bench and measure the volume of the PCR reaction. Transfer the sample to a skirted 384-well PCR plate.

3. Shake the Mag-Bind® E-Z Pure to resuspend any Mag-Bind® E-Z Pure particles that may have settled. Add 1.8 volumes Mag-Bind® E-Z Pure to each well.

PCR Reaction Volume (µL)	Mag-Bind® E-Z Pure (µL)
5	9
10	18
14	25

4. Pipet up and down 5-10 times or vortex for 30 seconds.

5. Let sit at room temperature for 1 minute.

6. Place the plate on a magnetic separation device to magnetize the Mag-Bind® E-Z Pure. Let sit at room temperature until the Mag-Bind® E-Z Pure is completely cleared from solution.

7. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® E-Z Pure.

8. Add 30 µL 70% ethanol to each well.

9. Let sit at room temperature for 1 minute. It is not necessary to resuspend the Mag-Bind® E-Z Pure.

10. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® E-Z Pure.

11. Repeat Steps 8-10 for a second 70% ethanol wash step.

12. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the Mag-Bind® E-Z Pure. Remove any residue liquid with a pipettor.

    Note: It is important to dry the Mag-Bind® E-Z Pure before elution. Residual ethanol may interfere with downstream applications.

    Optional: Incubating the plate at 37°C can speed up the evaporation.

13. Remove the plate from magnetic separation device.

14. Add 30 µL Elution Buffer (or 10 mM Tris pH 8.5, TE Buffer, 0.1 mM EDTA, or diH_2O) to each well.

15. Pipet up and down 20 times or vortex for 30 seconds.

16. Let sit at room temperature for 2-3 minutes.

17. Place the plate on a magnetic separation device to magnetize the Mag-Bind® E-Z Pure. Let sit at room temperature until the Mag-Bind® E-Z Pure is completely cleared from solution.

18. Transfer the cleared supernatant containing purified DNA to a new 384-well microplate and seal with non-permeable sealing film.

19. Store the plate at 2-8°C if storage is only for a few days. For long-term storage, samples should be kept at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **800-832-8896**.

Possible Problems and Suggestions

Problem	Cause	Solution
Low yield	Low PCR product yield	Increase the number amplification cycles for PCR
	Smaller PCR product size	Small PCR fragments normally give lower yield.
	Ethanol residue	During the drying step, remove any liquid from bottom of the well
	Particle loss during the procedure	Increase magnetization time. Aspirate slowly.
	DNA remains bound to beads	Increase elution volume
	Incomplete resuspension of the beads during elution	Vortex or pipet up and down to fully resuspend the beads.
Problem	Cause	Solution
Primer carryover	Insufficient wash of the particles	Wash the beads one more time with 70% ethanol.
Problem		Solution
Non-specific amplification products were not removed	The size of the non-specific amplification products are larger than 100 bp	Non-specific amplification products larger than 100 bp are not efficiently removed from PCR products.
Problem	Cause	Solution
Problems in downstream applications	Salt carryover	70% ethanol must be stored at room temperature.
	Ethanol carryover	Ensure the beads are completely dried before elution.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Magnetic Separation Device for 96-well Plates	MSD-01
Mag-Bind® E-Z Pure (50 mL)	M1380-01
Mag-Bind® E-Z Pure (500 mL)	M1380-02
Elution Buffer (100 mL)	PDR048
96-well Microplate (500 µL) (25/pk)	EZ9604-02
Multichannel Disposable Reservoirs (100/pk)	AC1331-01
Sealing Film (100/box)	AC1200-01