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Introduction

E-Z 96® MagBeads® Cycle-Pure Kit allows rapid and reliable isolation of PCR products with high yield. The system combines Omega Bio-Tek's proprietary chemistries with the reversible nucleic acid-binding properties of paramagnetic beads to eliminate excess nucleotides, primers, and small, nontargeted amplification products, such as primer dimers. This kit is designed for 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.

Overview

The MagBeads® paramagnetic particles technology provides a better solution for nucleic acid purification than centrifugation and vacuum based technologies. The product can be easily scaled up and it provides very user-friendly handling procedures. If using the E-Z 96® MagBeads® Cycle-Pure Kit for the first time, please read this booklet to become familiar with the procedures. PCR products are first mixed with a MagBeads® Particles Solution/MGC Binding Buffer/absolute ethanol master mix. PCR products then selectively bind to the MagBeads® particles. With just two rapid wash steps, trace contaminants such as nucleotides, primers and small, nontargeted 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.

Storage and Stability

All components of the E.Z.N.A.® MagBeads® Cycle-Pure Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C.

Kit Contents

Product Number	M1322-01	M1322-02
Purifications	4 x 96	24 x 96
MagBeads® Particles Solution	5 ml	6 x 5 ml
MGC Binding Buffer	25 ml	6 x 25 ml
MGW Wash Buffer	15 ml	60 ml
Elution Buffer	40 ml	4 x 60 ml
Instruction Manual	1	1

Before Starting

- Please read this entire booklet to become familiar with the E-Z 96® MagBeads® Cycle-Pure Kit procedures.
- Dilute MGW Wash Buffer Concentrate with ethanol as follows and **store at room temperature**.

M1322-01 Add 35 ml absolute (96%-100%) ethanol.

M1322-02 Add 140 ml absolute (96%-100%) ethanol to each bottle.

- Prepare a master mix consisting of MagBeads® Particles Solution/MGC Binding Buffer/absolute (96%-100%) ethanol. Add ethanol as follows and **store at room temperature**:

M1322-01 Add 70 ml absolute (96%-100%) ethanol.

M1322-02 Add 70 ml absolute (96%-100%) ethanol

NOTE: The above master mix is for PCR fragments >150 bp. Refer to Protocol II, below, for instructions for master mix preparation for PCR fragments ≤150 bp. If you are processing both large and very small fragments, for which you will need separate master mixes, the master mixes can be prepared separately. In such a case, you must prepare master mixes by ratios.

TIP: The MagBeads® particles will settle and bead together in their container. Shake or vortex the bottle containing the MagBeads® particles before use. After the master mix is prepared, check for beading before use. The MagBeads® particles can be resuspended by gently shaking or vortexing the container or by pipetting the solution up and down a few times. (IMPORTANT)

- Material to be Supplied by User
- 96-well PCR plate containing PCR samples (up to 100µl/well)
 - Absolute ethanol (96%-100%)
 - 80% ethanol (*for small fragments protocol only*)
 - Magnetic Separation Stand (Cat #MSTND-01)
 - Multichannel pipettor
 - Polypropylene reservoirs
 - 96-well collection plate (Note: the type of collection plate to be used depends on the type of Magnetic Separation Stand used. For OBI's MSTND-01, a 500µl collection plate is recommended (Cat #MP-500)

E-Z 96® MagBeads® Cycle-Pure Protocol

Note: This protocol is for PCR products >150 bp. See Protocol II for smaller fragments.

1. Read the manufacturer's instruction manual for the magnetic separation stand, if provided.
2. Place the 96-well PCR Plate on the bench and measure the volume of the PCR reaction.
3. Add 2 volumes of MagBeads® Particles Solution/MGC Buffer/ethanol master mix to each PCR reaction product.

Note: The MagBeads® particles will settle and bead together after about an hour. Check the master mix before use. If beading has occurred, resuspend particles by gently shaking or vortexing or by pipetting.

4. Mix each well by pipetting up and down 4-5 times, then incubate 1 minute at room temperature. Mix again by pipetting up and down 4-5 times.
5. Transfer the mixed samples to a 96-well collection plate. Place the plate onto a magnetic separation stand to magnetize the MagBeads® particles. Mix with pipette tips to ensure the complete capture of the beads on the sides of each well. Solution will be clear when beads have completely migrated toward the magnets.
6. Remove and discard the cleared supernatant.
7. Remove the collection plate containing the MagBeads® particles from the magnetic separation stand. Add 200µl of MGW Wash Buffer diluted with ethanol to each well.

8. Mix each well by pipetting up and down 4-5 times, then incubate 1 minute at room temperature. Mix again by pipetting up and down 4-5 times.
9. Place the plate onto the magnetic separation stand to magnetize the MagBeads[®] particles. Mix with pipette tips to ensure the complete capture of the beads on the sides of each well.
10. Remove and discard the cleared supernatant.
11. Remove the collection plate containing the MagBeads[®] particles from the magnetic separation stand. Add 100µl of MGW Wash Buffer diluted with ethanol to each well.
12. Mix each well by pipetting up and down 4-5 times. Incubate 1 minute at room temperature. Mix again by pipetting up and down 4-5 times.
13. Place the plate onto the magnetic separation stand to magnetize the MagBeads[®] particles. Mix with pipette tips to ensure the complete capture of the beads on the side of each well.
14. Remove and discard the cleared supernatant.
15. Allow the plate to dry on the magnetic separation stand for 5-10 minutes. Remove any liquid residue from the wells by pipetting.

Note: It is critical that the plate is dried completely before elution, as residual traces of ethanol can interfere with downstream applications.

16. After the plate has dried completely, remove it from the magnetic separation stand and add 50-100ul Elution Buffer or water to each well to elute DNA from the magnetic particles.
17. Mix each well by pipetting up and down 4-5 times. Incubate 1 minute at room temperature. Mix again by pipetting up and down 4-5 times.
18. Place the plate onto the magnetic separation stand to magnetize the MagBeads[®] particles. Mix with pipette tips to ensure the complete capture of the beads on the sides of each well.
19. Transfer the cleared supernatant containing purified DNA to a new 96-well collection plate.
20. Seal the plate with a non-permeable plate sealer and store the plate

at 4° C if storage is only for a few days. For long-term storage samples should be kept at -20° C.

Optimized Protocol for Small PCR Products (≤ 150 bp)

PCR products of size less than 150 bp can be successfully purified by following this protocol.

1. Prepare a master mix consisting of MagBeads[®] Particles Solution/MGC Binding Buffer/absolute (96%-100%) ethanol. Add ethanol as follows and **store at room temperature:**

M1322-01 Add 120 ml absolute (96%-100%) ethanol.

M1322-02 Add 120 ml absolute (96%-100%) ethanol

2. Follow Protocol I, Steps 1-6 on Page 4.
3. Remove the collection plate containing the MagBeads[®] particles from the magnetic separation stand. Add 200µl of 80% ethanol to each well.
4. Mix each well by pipetting up and down 4-5 times. Incubate 1 minute at room temperature. Mix again by pipetting up and down 4-5 times.
5. Place the plate onto the magnetic separation stand to magnetize the MagBeads[®] particles. Mix with pipette tips to ensure the complete capture of the beads on the sides of each well.
6. Remove and discard the cleared supernatant.
7. Repeat Steps 4-6 with 100µl of 80% ethanol.
8. Allow the plate to dry on the magnetic separation stand for 5-10 minutes. Remove any liquid residue from the wells by pipetting.

Note: It is critical that the plate is dried completely before elution, as residual traces of ethanol can interfere with downstream applications.

9. After the plate has dried completely, remove it from the magnetic separation stand and add 50-100ul Elution Buffer or water to each well for elute DNA from the magnetic particles.
10. Mix each well by pipetting up and down 4-5 times. Incubate 1 minute at room temperature. Mix again by pipetting up and down 4-5 times.
11. Place the plate onto the magnetic separation stand to magnetize the MagBeads® particles. Mix with pipette tips to ensure the complete capture of the beads on the sides of each well.
12. Transfer the cleared supernatant containing purified DNA to a new 96-well collection plate.
13. Seal the plate with a non-permeable plate sealer and store the plate at 4° C if storage is only for a few days. For long-term storage samples should be kept at -20° C.

Troubleshooting

Problem	Cause	Suggestions
Low yield	Low PCR product yield	Increase the number amplification cycles for PCR
	Smaller PCR product size	Small PCR fragments normally give lower yield. See page 6 for optimized protocol very small fragments
	Ethanol residue	During the drying step, remove any liquid from bottom of the well
	Particle loss during the procedure	Increase magnetization time. Aspirate more slowly
	DNA remains bound to beads	Increase elution volume to 200 µl
	Incompletely resuspension of the beads during elution	Fully suspend the beads by pipetting up and down.
Primer carryover	Insufficient wash of the particles	Wash the beads one more time with MGW Wash Buffer
Non-specific amplification products were not removed	The size of the non-specific amplification products are larger than 100bp.	Non-specific amplification products larger than 100bp are not efficiently removed from PCR products.
Problems in downstream applications	Salt carry-over.	Wash Buffer must be at room temperature.
	Ethanol carry-over	Ensure the beads are completely dried before elution