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Introduction

E.Z.N.A.® MagBeads® Cycle-Pure Kit allows rapid and reliable isolation of PCR products with high yield. The system uses the reversible nucleic acid-binding properties of paramagnetic beads with DNA to eliminate excess nucleotides, primers and small, nontargeted amplification products, such as primer dimers. This kit is designed for fully automated purification of PCR samples. Purified PCR fragments are eluted in water and can be used for microarrays, automated fluorescent DNA sequencing, restriction enzyme digestion and other applications.

Overview

Magnetic particles offer better solution for nucleic acid purification than centrifugation and vacuum based technologies. It can be easily scale up and provide easy handling procedures. If using the E.Z.N.A.® MagBeads® Cycle-Pure Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. PCR products are mixed with MGC Binding Buffer, and PCR products will selected to bind with MagBeads® particles. With just one rapid wash steps remove trace contaminants such as nucleotides, primers and small, nontargeted amplification products, pure DNA is eluted in water or low ionic strength buffer. 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 22ºC-25ºC.

Kit Contents

Product Number	M1322-01	M1322-02
Purification	4 x 96	24 x 96
MagBeads® Particles Solution C	420 µ l	2.7 ml
MGC Binding Buffer	30 ml	75 ml
MGW Wash Buffer	60 ml	6 × 60 ml
Elution Buffer	40 ml	4 × 60 ml
Instruction Booklet	1	1

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.® MagBeads® Cycle-Pure Kit procedure.

- Dilute MGW Wash Buffer Concentrate with ethanol as follows and **store at room temperature**.

M1322-01	Add 140 ml absolute ethanol.
M1322-02	Add 140 ml absolute ethanol to each bottle.

- Prepare the MGC Binding Buffer by adding ethanol as following:

M1322-01	Add 70 ml absolute (100%) ethanol.
M1322-02	Add 175 ml absolute (100%) ethanol

Material to be Supplied by User

- 96-well PCR plate containing PCR samples (up to 100µl/well)
- Absolute ethanol (96%-100%)
- 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# MC-2001)

MagBead™ Cycle-Pure Protocol

Note: The following protocol are based on the assumption that the maximum volume of PCR reaction for a 96-well plate is 100µl. For large volume of PCR reaction, adjust the volume proportionately.

1. Read the instruction of the magnetic separation device from the manufacture.

2. Place the 96-well PCR Plate on the bench and measure the volume of PCR reaction.

3. Resuspend the Magbead™ Particles Solution C throughly by shaking or pipetting. Add 3ul of Magbead™ Particles Solution for each PCR reaction.

4. Add 2 volume MGC Buffer diluted with ethanol.

   NOTE: The MagBeads® Particles will settle and bead together at the bottom of their container after several hours. Please check container before use. If beading has occurred, gently shake or vortex container until particles have been redispersed in solution. (IMPORTANT)

   Tip: MGC Binding Buffer and MagBeads® Particles Solution C can be combined in appropriate proportions to make a master mix before starting the procedure. Add 200 µl per well of MagBeads® Particle Solution/MGC Binding Buffer master mix to each well of the 100ul PCR reaction.

5. Mix each well by pipetting up and down 4-5 times. Incubate 1 minutes at room

temperature.

6. Transfer the mixed samples to a 96-well collection plate. Place the plate onto a magnetic separation device to magnetize the Magbead™ particles. Mix with pipette tips to ensure the complete capture of the beads to the side of each well.

7. **Remove and discard the cleared supernatant.**

8. Remove the collection plate containing the Magbead™ particles from the magnetic separation device. Add 200µl of MGW Wash Buffer diluted with ethanol to each well.

9. Mix each well by pipetting up and down 4-5 times. Incubate 1 minutes at room temperature. Repeating the mix by pipetting up and down 4-5 times.

10. Place the plate onto a magnetic separation device to magnetize the Magbead™ particles. Mix with pipette tips to ensure the complete capture of the beads to the side of each well.

11. **Remove and discard the cleared supernatant**.

12. Remove the collection plate containing the Magbead™ particles from the magnetic separation device. Add 200µl of MGW Wash Buffer diluted with ethanol to each well.

13. Mix each well by pipetting up and down 4-5 times. Incubate 1 minutes at room temperature. Repeating the mix by pipetting up and down 4-5 times.

14. Place the plate onto a magnetic separation device to magnetize the Magbead™ particles. Mix with pipette tips to ensure the complete capture of the beads to the side of each well.

15. **Remove and discard the cleared supernatant**.

16. Leave the plate to dry on the magnetic separation device for 5-10 minutes. Remove any residue liquid from well by pipetting.

17. Remove the plate from magnetic separation device. Add 50-100ul Elution Buffer or water to each well for elute DNA from magnetic particles.

18. Mix each well by pipetting up and down 4-5 times. Incubate 1 minutes at room temperature. Repeating the mix by pipetting up and down 4-5 times.

19. Place the plate onto a magnetic separation device to magnetize the Magbead™ particles. Mix with pipette tips to ensure the complete capture of the beads to the side of each well.

20. Transfer the cleared supernatant containing purified DNA to a new 96-well collection plate.

21. Seal the plate with a nonpermeable plate sealer and store the plate at 4° C for few days and at -20° C for long term storage.

Optimize Protocol for small size PCR product (≤150 bp)

PCR products with size less than 150bp can be more efficiently purified by adjust the binding and washing conditions.

1. Prepare the master mix of Magbead™ Particles solution/MGC Buffer by combining Magbead Particle Solution and MGC Binding Buffer. Add ethanol as follows and **store at room temperature**.

   M1322-01 Add 120 ml absolute ethanol.

   M1322-02 Add 300 ml absolute ethanol

2. Follow the regular protocol step 1-7 on page 4.

3 Remove the collection plate containing the Magbead™ particles from the magnetic separation device. Add 200µl of 70% ethanol to each well.

4. Mix each well by pipetting up and down 4-5 times. Incubate 1 minutes at room temperature. Repeating the mix by pipetting up and down 4-5 times.

5. Place the plate onto a magnetic separation device to magnetize the Magbead™ particles. Mix with pipette tips to ensure the complete capture of the beads to the side of each well.

6. **Remove and discard the cleared supernatant**.

7. Repeat the step 4-6 with 100µl 70% ethanol.

8. Leave the plate to dry on the magnetic separation device for 5-10 minutes. Remove any residue liquid from well by pipetting.

9. Remove the plate from magnetic separation device. Add 50-100ul Elution Buffer or water to each well for elute DNA from magnetic particles.

10. Mix each well by pipetting up and down 4-5 times. Incubate 1 minutes at room temperature. Repeating the mix by pipetting up and down 4-5 times.

11. Place the plate onto a magnetic separation device to magnetize the Magbead™ particles. Mix with pipette tips to ensure the complete capture of the beads to the side of each well.

12. Transfer the cleared supernatant containing purified DNA to a new 96-well collection plate.

13. Seal the plate with a nonpermeable plate sealer and store the plate at 4° C for few days and at -20° C for long term storage.

Troubleshooting

Problem	Cause	Suggestions
Lower yield	Lower PCR yield	Increase the number amplification cycle for PCR
	Smaller PCR product size	small PCR product normally give lower yield. See page 6 for optimized protocol
	Residue of ethanol	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
Non-specific amplification products were not removed	The size of the non-specific amplification products are larger than 100bp.	Non-specific amplification products large 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