EcoExtra™ Magnetic Beads PCR purification kit Protocol

For research use only

Catalogue Number

MCP004, MCP048, MCP096

Introduction

The EcoExtra™ Magnetic Beads PCR Purification Kit employs advanced paramagnetic bead technology for the efficient purification of PCR amplicons. This system utilizes an optimized buffer to selectively bind DNA fragments of 100 bp or larger to paramagnetic beads. The protocol consists of three key steps: binding, washing, and elution, which collectively remove primers, nucleotides, salts, and enzymes from the reaction mixture, yielding a highly purified PCR product. The purified DNA can then be used in various downstream applications, including sequencing, cloning, genotyping, and library construction for next-generation sequencing (NGS). **More important, the EcoExtra™ series kits enable up to 80% reduction of plastic material waste compared to silica-based kits. Make lab works a lot more eco-friendly**.

Components

	MCP004	MCP048	MCP096
MCP Magnetic bead[1]	200 µl	2.5 ml	5 ml
Wash Buffer[2] (Add Ethanol)	1 ml (4 ml)	5 ml (20 ml)	10 ml (40 ml)
Elution Buffer	1 ml	2 ml	6 ml

[1] Store at 2-8°C upon arrival, for up to 12 months. Thoroughly shake the reagent prior to use. It should have a homogeneous appearance and uniform color.

[2] Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements

- 8-strip PCR tube, 96-well PCR reaction plate.
- Magnetic plate for 96-well PCR reaction plate, Magnetic stand for 8-strip PCR tube (Geneaid EcoMag Stand-8, TZ008).

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Important before use

- Allow MCP Magnetic bead to reach room temperature.
- Gently shake the reagent prior to use. It should have a homogeneous appearance and uniform color.

1. DNA Binding

- Shake the **MCP Magnetic bead** to resuspend any magnetic particles that may have settled. Add **45 µl of MCP Magnetic bead** to each well of the 8-strip PCR tube/reaction plate containing 25 µl of PCR product.

NOTE: If the volume of the PCR product is less than 25 µl, add an appropriate volume of ddH_2O to bring the final volume to 25 µl.

- Thoroughly mix the reagent and sample by pipetting up and down 10 times. Allow the mixed samples to incubate at room temperature for 5 minutes to ensure optimal recovery. This step facilitates the binding of DNA fragments that are 100 bp or larger to the magnetic beads.
- Place the 8-strip PCR tube/reaction plate onto an appropriate 8-strip PCR tube Magnetic Stand/96 well Magnetic Plate for 3 minutes or until the solution clears.

NOTE: It is crucial to wait until the solution is clear before proceeding with the washing step; otherwise, beads may be lost, resulting in low DNA recovery.

- This step must be carried out with the 8-strip PCR tube/reaction plate placed on the 8-strip PCR tube Magnetic Stand/96 well Magnetic Plate. Aspirate the cleared solution from the 8-strip PCR tube/reaction plate and discard it, leaving 5 µl of supernatant behind. Failure to do so may result in the loss of beads along with the supernatant.

NOTE: Be careful **DO NOT** disturb the separated magnetic beads.

2. Wash

- This step must be carried out with the 8-strip PCR tube/reaction plate placed on the 8-strip PCR tube Magnetic Stand/96 well Magnetic Plate. Dispense **200 µl of Wash Buffer (make sure ethanol was added)** into each well of the 8-strip PCR tube/reaction plate and incubate for 30 seconds at room temperature. Then, aspirate the Wash Buffer and discard it.
- Repeat for a total of two washes.

NOTE: The beads do not easily dislodge in Wash Buffer, so it is not necessary to leave any supernatant behind. Ensure all traces of Wash Buffer are removed because residual ethanol may interfere with downstream applications.

- Allowing the 8-strip PCR tube/reaction plate to stand at room temperature for 5 minutes will help ensure that any residual ethanol has evaporated. Take care not to let the beads dry out completely, as this will significantly reduce elution efficiency.

3. Elution

- Remove the 8-strip PCR tube/reaction plate from the magnetic stand, add **40 µl of Elution Buffer** to each well of the 8-strip PCR tube/reaction plate and mix thoroughly by pipetting up and down 10 times. Incubate for 2 minutes.

NOTE: At a 40 µl elution volume, the liquid level will be sufficient to contact the magnetic beads. More than 40 µl of elution buffer can be used if a larger end volume is required, but the final concentration will be more diluted. While using less than 40 µl will necessitate additional mixing to ensure contact with the beads and may not be adequate to elute the entire PCR product.

- Place the 8-strip PCR tube/reaction plate on the 8-strip PCR tube Magnetic Stand/96 well Magnetic Plate for 1 minute to separate the beads from the solution. The supernatant now contains the eluted DNA. The beads may be discarded.
- Transfer the supernatant to a fresh tube/plate for storage and analysis.

Troubleshooting

Low Yield

Bead loss.

- If beads are aspirated into the tips during the removal of the supernatant, any nucleic acid bound to these beads will also be lost. Therefore, aspirate slowly and carefully to remove the initial supernatant without disturbing the beads.

Insufficient mixing.

- Through mixing during the initial binding and elution steps is essential. During elution, ensure that 40 µl of Elution Buffer is used. It is also important to maintain appropriate incubation times to allow sufficient binding or dissociation of the nucleic acids with the beads. Vortexing during the binding step may be inefficient due to the sample's viscosity.

Incomplete Purification Problems

Primer carryover.

- The final concentration of the binding buffer in the MCP Magnetic bead determines the size of the DNA fragments that will bind to the beads. MCP Magnetic bead is designed to capture PCR products larger than 100 bp while removing primers shorter than 50 bases. However, larger primers and primer dimers can still adhere to the beads. Ensure that the correct volume of reagent is added and that the PCR product has not evaporated to a smaller volume during cycling.

Residual ethanol contamination.

- If your downstream enzymatic reaction is highly sensitive to trace amounts of ethanol, incorporate a conservative drying step of two minutes. Be aware that large fragments can bind tightly to the beads, making them difficult to elute after they have fully dried onto the beads.