Presto™ PureXP Magnetic Beads for DNA Cleanup

For research use only

Catalogue Number

MXP004s, MXP005, MXP060, MXP450

Introduction

The Geneaid Presto™ PureXP Magnetic Beads utilizes unique paramagnetic bead technology for high-throughput purification of PCR amplicons. This system uses an optimized buffer to selectively bind DNA fragments of 100 bp or larger to paramagnetic beads. The protocol consists of binding, washing, and elution steps, effectively removing primers, nucleotides, salts, and enzymes from the reaction mixture, resulting in a more purified PCR product. The purified products can be used for downstream applications, such as PCR, sequencing, cloning, genotyping and library construction for NGS.

Components

	MXP004s	MXP005	MXP060	MXP450
Presto™ PureXP Magbead*	400 µl	5 ml	60 ml	450 ml

* 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.

Specifications

The Presto™ PureXP Magbead can be utilized for PCR purification in 8-strip PCR tubes, 96-well and 384-well formats. The following tables display the number of PCR reactions that the Presto™ PureXP Magbead can purify based on reaction volumes. Typical reaction volume is 10–20 µl for the 8-strip PCR tubes and 96-well plate and 5–10 µl for the 384-well plate.

Number of PCR reactions purified based on reaction volume

PCR reaction volumes (µl)	MXP004s	MXP005	MXP060	MXP450
5 µl	44 rxns	555 rxns	6666 rxns	50000 rxns
10 µl	22 rxns	277 rxns	3333 rxns	25000 rxns
20 µl	11 rxns	138 rxns	1666 rxns	12500 rxns
50 µl	4 rxns	55 rxns	666 rxns	5000 rxns
100 µl	2 rxns	27 rxns	333 rxns	2500 rxns

Additional Requirements

Consumables and Hardware

- 8-strip PCR tube, 96-well PCR reaction plate, 384-well PCR reaction plate.
- 300 µl/ 1.2 ml deep well plate (optional).
- Magnetic plate for 96/ 384-well PCR reaction plate, Magnetic stand for 300µl/ 1.2 ml deep well plate (Geneaid 96 Magnetic Stand, MNS096-W), Magnetic stand for 8-strip PCR tube (Geneaid EcoMag Stand-8, TZ008).

Reagents

- Fresh 70% ethanol.

NOTE: 70% ethanol should be prepared prior to use for optimal results. When preparing 70% ethanol, it is suggested to measure 70 ml of ethanol and 30 ml of water separately, then combining and mixing them will generate ~95 ml of 70% ethanol.

- Elution Buffer (10mM Tris, pH 8.0), sterile water or TE Buffer (10mM Tris, 1 mM EDTA, pH 8.0) for DNA elution (Elution Buffer and TE Buffer can be purchased from Geneaid).

Important before use

- Allow Presto™ PureXP Magbead to reach room temperature.
- Gently shake the reagent prior to use. It should have a homogeneous appearance and uniform color.

96 Well Format Procedure

1. Assess the necessity of a plate transfer

- If the PCR reaction volume multiplied by 2.8 exceeds the capacity of the PCR plate, a transfer to a 300 µl or a 1.2 ml deep-well plate is required.

2. DNA Binding

- Shake the Presto™ PureXP Magbead to resuspend any magnetic particles that may have settled. Add Presto™ PureXP Magbead according to the PCR volume table below:

PCR reaction volumes (µl)	Presto™ PureXP Magbead volume at 1.8x (µl)
10 µl	18 µl
20 µl	36 µl
50 µl	90 µl

NOTE: The volume of Presto™ PureXP Magbead for a given reaction can be calculated using the following equation: (Volume of Presto™ PureXP Magbead per reaction) = 1.8 × (PCR Volume).

- 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 reaction plate onto an appropriate 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 reaction plate placed on the 96 well Magnetic Plate. Aspirate the cleared solution from the 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.

3. Wash

- This step must be carried out with the reaction plate placed on the 96 well Magnetic Plate. Dispense 180µl of 70% ethanol into each well of the reaction plate and incubate for 30 seconds at room temperature. Then, aspirate the ethanol and discard it.
- Repeat for a total of two washes.

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

- Allowing the 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.

4. Elution

- Remove the reaction plate from the magnetic plate, add 40 µl of Elution Buffer, sterile water or TE Buffer to each well of the 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 reaction plate onto the 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 plate for storage and analysis.

384 Well Format Procedure

1. DNA Binding

- Shake the Presto™ PureXP Magbead to resuspend any magnetic particles that may have settled. Add Presto™ PureXP Magbead according to the PCR volume table below:

PCR reaction volumes (µl)	Presto™ PureXP Magbead volume at 1.8x (µl)
5 µl	9 µl
7 µl	12.6 µl
10 µl	18 µl
14 µl	25.2 µl

NOTE: The volume of Presto™ PureXP Magbead for a given reaction can be calculated using the following equation: (Volume of Presto™ PureXP Magbead per reaction) = 1.8 × (PCR Volume).

- 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 reaction plate onto an appropriate 384 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 reaction plate placed on the 384 well Magnetic Plate. Aspirate the cleared solution from the reaction plate and discard it, leaving a few 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 reaction plate placed on the 384 well Magnetic Plate. Dispense 30 µl of 70% ethanol into each well of the reaction plate and incubate for 30 seconds at room temperature. Then, aspirate the ethanol and discard it.
- Repeat for a total of two washes.

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

- Allowing the plate to stand at room temperature for at least 1 minute 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 reaction plate from the magnetic plate, add 30 µl of Elution Buffer, sterile water or TE Buffer to each well of the reaction plate and mix thoroughly by pipetting up and down 10 times. Incubate for 2 minutes.

NOTE: At a 30 µl elution volume, the liquid level will be sufficient to contact the magnetic beads. More than 30 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 15 µl will necessitate additional mixing to ensure contact with the beads and may not be adequate to elute the entire PCR product.

- Place the reaction plate onto the 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 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 the minimum elution volume—40 µl for the 96-well format and 15 µl for the 384-well format 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.

Large reaction volume.

- Reactions with large volumes can benefit from extended binding and separation times. Increase the binding duration to 10 minutes, and ensure that all beads are fully separated before removing the supernatant.

Low ethanol concentration.

- Ethanol must be at least 70% and ethanol should be prepared prior to use for optimal results. Stock ethanol can absorb moisture from the atmosphere over time, resulting in a decreased concentration.

Low elution volume.

- A small elution volume results in reduced recovery, as some elution buffer invariably remains on the beads' surface. This residual volume is influenced by the well shape and the quantity of beads present, meaning that a smaller elution volume will leave a higher percentage of the eluate behind.

Incomplete Purification Problems

Primer carryover.

- The final concentration of the binding buffer in the Presto™ PureXP Magbead determines the size of the DNA fragments that will bind to the beads. Presto™ PureXP Magbead 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. Additionally, rinse the well with ethanol in any areas where the sample has contacted the plastic during mixing and binding. If excess primer is present during purification, reducing the amount of primer used in the PCR reaction can also be advantageous.

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.