MAGBIO®

ACCELERATING genomic research

HighPrep™ PCR

Efficient cleanup for post PCR and NGS library construction

Catalog Nos. AC-60005, AC-60050, AC-60250, AC-60500

Manual Revision v1.09

- Magnetic beads based chemistry
- No centrifugation or filtration

PROTOCOL

Contents

For Research Use Only. Not for use in diagnostic procedures.

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Product Description

The HighPrep™ PCR post PCR clean up system is based on paramagnetic bead technology designed for an efficient purification of amplicons. The purification consists of removal of salts, primers, primer-dimers, dNTPs, as DNA fragments are selectively bound to the magnetic beads particles; and highly purified DNA is eluted with low salt elution buffer or water which can be used directly for downstream applications. This protocol can be used for manual procedure as well as guideline for adapting the kit to automatic liquid handling instruments. For availability of ready-to-run scripts please contact MagBio Genomics.

Amplicons purified with the HighPrep PCR system are ready to be used in the following applications:

- PCR
- Mutation detection and genotyping
- Sequencing (Sanger and Next Generation)
- Microarrays
- Restriction enzyme clean up
- Cloning

Process

HighPrep™ PCR uses a simple 3 steps procedure: Bind-Wash-Elute. HighPrep™ PCR is added to the PCR reaction sample. The protocol utilizes a magnet plate (magnet stand) for processing the PCR reaction sample. During the process, contaminants and salts are washed off and pure DNA is eluted, ready to be used in subsequent applications.

Product Specifications

Product Number	Description	Number of Reactions	Storage Conditions
AC-60005	HighPrep™ PCR - 5 mL	278	4-8°C **DO NOT FREEZE**
AC-60050	HighPrep™ PCR - 50 mL	2,778	
AC-60250	HighPrep™ PCR - 250 mL	13,890	
AC-60500	HighPrep™ PCR - 500 mL	27,780	

Number of reactions is based on typical 10µL PCR reaction volume.
Volume of HighPrep™ PCR reagent per reaction = 1.8 x (PCR Reaction Volume)

Materials Supplied in the Kit

- HighPrep™ PCR paramagnetic beads solution
- Store at 4°C. DO NOT FREEZE. HighPrep PCR is stable for 12 months when stored at 4°C.
- Thoroughly shake the HighPrep™ PCR reagent to resuspend the beads before use.

Equipment and Reagents to Be Supplied by User:

- 80% ethanol (Prepared from non denatured ethanol)
- 10mM TRIS-HCL pH 8.0 (DNA Elution)
- Reagent grade water
- 1mM EDTA

Magnet (Stand and Plate):

For 1.5mL tube format: MagBio MagStand10 - Magnet Stand (1.5ml x 10)
MagBio Genomics, Inc., Cat# MBMS-10, www.magbiogenomics.com
For 96 well format: 96 well ring plate
For 384 well format: 384 magnet plate

Reaction Plate:

For 96 well format: 96 well cycling plate
For 384 well format: 384 well cycling plate

HighPrep™ PCR - 96 well protocol

*** Bring the HighPrep PCR to room temperatue for at least 30 min before use.**

1. **Shake thoroughly the HighPrep PCR reagent to fully resuspend the magnetic beads.**

2. **Transfer PCR reaction to appropriate 96-well plate.**
   For 50µl reaction, adjust volume using sterile water.

3. **Add HighPrep PCR reagent volume according to the PCR reaction.**
   See table below to determine appropriate volume.

PCR Reaction Volume (µL)	HighPrep PCR Volume at 1.8X (uL)*
10	18
20	36
50	90

* Formula used to calculate the volume of HighPrep PCR reagent needed for PCR reaction:
HighPrep PCR reagent volume per reaction = 1.8 X PCR reaction volume.

4. **Mix thoroughly the HighPrep PCR reagent and PCR sample by mix pipetting up and down 6-8 times.**

5. **Incubate the mixture for 5 minutes at room temperature.**

6. **Place the sample plate on the 96 magnetic separation device for 3 minutes or until the solution clears.** Beads will pull to the side of the well.

7. **With the sample plate still on the magnet, remove and discard the supernatant by pipetting.**

   ⚠ Do not disturb the attracted beads while aspirating the supernatant.

8. **With the sample plate on the magnet, add 200 µl of 80% ethanol to each well and incubate for 30 seconds at room temperature.**

9. **With the plate still on the magnet, remove and discard the supernatant by pipetting.**

10. **Repeat steps 8-9 for a total of two 80% ethanol washes.**

11. **Dry the beads by incubating the plate for 10-15 minutes at room temperature with the plate still on the magnetic separation device.**

    ⚠ It is critical to completely remove all traces of alcohol but take caution in not over drying the beads as this will reduce the yield.

12. **Remove the sample plate from the magnetic separation device. Add 40µl of elution buffer (reagent grade water, TRIS-HCl pH 8.0 or TE buffer) to each well and pipet up and down 5 times to mix.** Prewarming the elution buffer at 55°C can increase the yield.

13. **Incubate for 2 minutes at room temperature.**

14. **Place the sample plate back on the magnetic separation device and wait 1 minute or until the magnetic beads clear from solution.**

15. **Transfer the eluate (cleared supernatant) to a new plate for storage or for subsequent applications.**

HighPrep™ PCR - 384 Well Format

1. **Shake thoroughly the HighPrep PCR reagent to fully resuspend the magnetic beads.**

2. **Transfer PCR reaction to appropriate 384-well plate.**
   For 50µl reaction, adjust volume using sterile water.

3. **Add HighPrep PCR reagent volume according to the PCR reaction.**
   See table below to determine appropriate volume.

PCR Reaction Volume (µL)	HighPrep PCR Volume at 1.8X (uL)*
5	9
7	12.6
10	18

* Formula used to calculate the volume of HighPrep PCR reagent needed for PCR reaction:
HighPrep PCR reagent volume per reaction = 1.8 X PCR reaction volume.

4. **Mix thoroughly the HighPrep PCR reagent and PCR sample by mix pipetting up and down 6-8 times.**

5. **Incubate the mixture for 5 minutes at room temperature.**

6. **Place the sample plate on the 384 magnetic separation device for 1 minute or until the solution clears.** Beads will pull to the side of the well.

7. **With the sample plate still on the magnet, remove and discard the supernatant by pipetting.**

   ⚠ Do not disturb the attracted beads while aspirating the supernatant.

8. **With the sample plate on the magnet, add 30 µl of 80% ethanol to each well and incubate for 30 seconds at room temperature.**

9. **With the plate still on the magnet, remove and discard the supernatant by pipetting.**

10. **Repeat steps 8-9 for a total of two 80% ethanol washes.**

11. **Dry the beads by incubating the plate for 3-5 minutes at room temperature with the plate still on the magnetic separation device.**

    ⚠ It is critical to completely remove all traces of alcohol but take caution in not over drying the beads as this will reduce the yield.

12. **Remove the sample plate from the magnetic separation device. Add 30µl of elution buffer (reagent grade water, TRIS-HCl pH 8.0 or TE buffer) to each well and pipet up and down 5 times to mix.** Prewarming the elution buffer at 55°C can increase the yield.

13. **Incubate for 2 minutes at room temperature.**

14. **Place the sample plate back on the magnetic separation device and wait 1 minute or until the magnetic beads clear from solution.**

15. Transfer the eluate (cleared supernatant) to a new plate for storage or for subsequent applications.

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