MAGBIO

"WE MAKE NGS BETTER"

HighPrep™ PCR

Efficient clean-up for post PCR and fragment size-selection for NGS library construction

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

Manual Revision v2.0

- Magnetic beads based chemistry
- No centrifugation or filtration
- Efficient clean-up
- Precise size-selection

PROTOCOL

Contents

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

Information in this document is subject to change without notice.

Product Description

The HighPrep™ PCR clean-up system is based on paramagnetic bead technology designed for efficient purification of amplicons and size-selection of DNA fragments in library preparation for NGS. The purification consists of removal of salts, primers, primer-dimers, and dNTPs. DNA fragments are selectively bound to the magnetic bead 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 automated 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	2-8°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 2-8°C. DO NOT FREEZE. HighPrep™ PCR is stable for 14 months when stored at 2-8°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 MagStand12 - Magnet Stand (1.5ml x 12)
MagBio Genomics, Inc., Cat# MBMS-12, www.magbiogenomics.com
For 96 well format: 96 well cycling plate
MagBio Magnetic Separation Device - (96 well microplate format)
MagBio Genomics, Inc., Cat# MYMAG-96, www.magbiogenomics.com
MagBio Genomics, Inc., Cat# MYMAG-96X www.magbiogenomics.com
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 clean-up system - 96 well protocol

⚠ Bring the **HighPrep™ PCR** to room temperature 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 50 µl 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 the **HighPrep™ PCR** reagent and PCR sample by 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 40 µl 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 to 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 3 minutes or until the magnetic beads clear from the solution.

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

HighPrep™ PCR clean-up system - 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 50 µl 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

* 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 the **HighPrep™ PCR** reagent and PCR sample by 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 2 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 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 30 µl 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 to 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 2 minutes or until the magnetic beads clear from the solution.

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

DNA Size-Selection

To obtain a custom protocol for DNA size-selection of a specific fragment size, contact support@magbiogenomics.com.

Ordering and Related Product Information

Post PCR and Next Gen library prep clean-up system

Catalog No.	Product
RC-90005	HIghPrep™ RNA Elite (5 mL)
RC-90050	HIghPrep™ RNA Elite (50 mL)
RC-90250	HIghPrep™ RNA Elite (250 mL)
RC-90500	HIghPrep™ RNA Elite (500 mL)

Whole blood stabilization tubes

Catalog No.	Product	Description
BS-CF10-100	Blood STASIS-ccfDNA 9 mL (100)	100 tubes: 2 ml Additive, 7 ml blood draw volume
BS-CF6-100	Blood STASIS-ccfDNA 6 mL (100)	100 tubes: 1.5 ml Additive, 4.5 ml blood draw volume
BS-CF3-200	Blood STASIS-ccfDNA 3 mL (200)	200 tubes: 0.5 ml Additive, 2.5 ml blood draw volume
BS-DR10-100	Blood STASIS-DNA/RNA 9 mL (100)	100 tubes: 2 ml Additive, 7 ml blood draw volume
BS-DR6-100	Blood STASIS-DNA/RNA 6 mL (100)	100 tubes: 1.5 ml Additive, 4.5 ml blood draw volume
BS-DR3-200	Blood STASIS-DNA/RNA 3 mL (200)	200 tubes: 0.5 ml Additive, 2.5 ml blood draw volume

gDNA Isolation Kit

Catalog No.	Product	Description	Preps
HPBTS-D96	HighPrep™ Blood & Tissue DNA Kit	Genomic DNA isolation from 20-250 µl of blood, lysate of tissues, mouse tails, cultured cells, or buccal swabs	96
HPBTS-D96X4	HighPrep™ Blood & Tissue DNA Kit	Genomic DNA isolation from 20-250 µl of blood, lysate of tissues, mouse tails, cultured cells, or buccal swabs	384

Magnetic Separation Devices

Catalog No.	Description
MYMAG-96	Handheld Magnetic Separation Device (96 well microplate format)
MYMAG-96X	Magnetic Separation Device (96 well ring format)
MBMS-12	MagStrip magnetic stand (1.5 mL x 12)
MBMS-31550	15ml and 50ml magnetic stand combo. (3x15ml and 3x50ml)

www.magbiogenomics.com