MAGBIO
"WE MAKE NGS BETTER"

HighPrep™ PCR

INSTRUCTIONS FOR USE

Catalog Number:
AC-60001, AC-60005, AC-60050, AC-60100, AC-60250, AC-60500

Revision v1.0

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For descriptions of symbols on product labels or product documents, go to **https://www.magbiogenomics.com/.**

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the kit.

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1 Product information

Intended use.

HighPrep™ PCR Kit uses a magnetic bead-based chemistry for cleaning up DNA amplicons and binding of target DNA fragment of the desired size range. The kit is intended for robust and efficient amplicon clean-up and DNA fragment selection in next generation sequencing library preparation.

Product information

HighPrep™ PCR (Cat. No. AC-60005, AC-60050, AC-60250, and AC-60500) is specifically designed for selection of sheared DNA fragments and amplicon purification. Fragment size selection by HighPrep™ PCR is always tight and consistent. The purification process consists of removal of salts, primers, primer-dimers, dNTPs, salts, enzymes, adapters, and adapter dimers. DNA amplicons and 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. HighPrep™ PCR is compatible with most next generation sequencing platforms.

Contents and storage of the Kit

Product Catalog Number	Description	Number of Reactions (based on 1.8x ratio)	Storage Conditions
AC-60005	HighPrep™ PCR - 5 mL	278	2-8°C DO NOT FREEZE
AC-60050	HighPrep™ PCR - 50 mL	2,778	
AC-60100	HighPrep™ PCR - 100 mL	5,556	
AC-60250	HighPrep™ PCR - 250 mL	13,890	
AC-60500	HighPrep™ PCR - 500 mL	27,780	

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

Kit Stability

HighPrep™ PCR is stable for 18 months from date of manufacture when stored at 2-8°C.

Required materials not supplied

The following materials are needed but not supplied with the kit:

Item	Source
80% Ethanol (Prepared from non-denatured ethanol)	Any vendor of choice
10mM TRIS-HCL pH 8.0, Reagent grade water, or 1mM EDTA (DNA Elution)	Any vendor of choice
Magnetic stand or plate (manual DNA recovery)	www.magbiogenomics.com
Automated platforms for magnetic bead purification (Automated DNA recovery)	Compatible with most platforms but the customer must validate the protocol.
96 well PCR plate or 384 well PCR plate or 1.5 mL-2 mL Tubes (for sample processing)	www.nrsbiologics.com or thermofisher.com
Laboratory mixer, vortex, or equivalent	Any laboratory vortex that can mix the beads efficiently.
Single and multichannel adjustable pipettors (1.00 µL to1000 µL)	Any accurate pipette that has been calibrated
Cold block or ice	Any vendor of choice
Sterile aerosol barrier (filtered) pipette tips (DNase and RNase Free)	Any vendor of choice

2 HighPrep™ PCR - 96 well or 1.5 - 2 mL Tube Clean-Up Protocol

***Bring HighPrep™ PCR to room temperature for at least 30 mins before use**

1. Shake the HighPrep™ PCR reagent thoroughly to fully resuspend the magnetic beads.
2. Transfer PCR reaction to appropriate 96 well PCR plate or 1.5 - 2 mL RNase-DNase free tube.

   For 50 µl reaction, adjust volume using sterile water.
3. Add HighPrep™ PCR reagent volume according to the PCR reaction table below:

   See table below to determine appropriate volume.

PCR Reaction Volume (µl)	HighPrep™ PCR Volume at 1.8X (µl)*
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. Thoroughly mix 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/tube on the 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/tube still on the magnet, remove and discard the supernatant by pipetting.

   Note: 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.

    Note: 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.

    Note: 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 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.

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HighPrep™ PCR – 384 well Clean-Up Protocol

***Bring HighPrep PCR to room temperature for at least 30 mins before use**

1. Shake the HighPrep™ PCR reagent thoroughly 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 table below:
   See table below to determine appropriate volume.

PCR Reaction Volume (µl)	HighPrep™ PCR Volume at 1.8X (µl)*
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. Thoroughly mix 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 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.
   Note: 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.
    Note: 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.
    Note: 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 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.

2 HighPrep™ PCR – DNA Fragment Selection

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

3 Safety and Warning Information

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the “Product Resource” tab when viewing the product kit**.**
Download SDS at www.magbiogenomics.com.

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Warning and Precautions

- This kit has not been FDA cleared or approved and it is not used for diagnosing a patient.
- Care should be taken to avoid contamination by adequately controlling sample preparation, handling, and processing.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.
- Specimens may be infectious. Follow Universal Precautions when handling specimens.