MAGBIO

"WE MAKE NGS BETTER"

HighPrep PCR - DX

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

Catalog Nos. AC-60001E, AC-60005E, AC-60050E, AC-60250E, AC-60500E
Manual Revision 3.1
WI-72-62

- Magnetic bead-based chemistry
- No centrifugation or filtration
- Efficient cleanup
- Precise size-selection

Instructions For Use

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For *in vitro* diagnostic procedures.

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Product Description

HighPrep PCR - DX 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 a 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 are ready to be used in the following applications:

- PCR
- Mutation detection and genotyping
- Next generation sequencing
- Microarrays
- Restriction enzyme digests
- Cloning

Process

HighPrep PCR - DX uses a simple 3 steps procedure: Bind-Wash-Elute. HighPrep PCR - DX 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

Catalog Number	Description	Number of Reactions	Storage Conditions
AC-60001E	HighPrep PCR - DX (1 mL) (Sample size)	55	2-8°C DO NOT FREEZE
AC-60005E	HighPrep PCR - DX (5 mL)	277	
AC-60050E	HighPrep PCR - DX (50 mL)	2,777	
AC-60250E	HighPrep PCR - DX (250 mL)	13,888	
AC-60500E	HighPrep PCR - DX (500 mL)	27,777	

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

Storage and Preparation

- Store at 2-8°C. **DO NOT FREEZE.** HighPrep PCR - DX is stable for 18 months when stored at 2-8°C.
- Keep at room temperature for 30 minutes prior to use.
- Thoroughly shake the HighPrep PCR - DX to resuspend the beads before use.

Materials Supplied in the Kit

- HighPrep PCR - DX

Equipment and Reagents to Be Supplied by the User:

- 80% Ethanol (Prepared from non denatured Ethanol)
- Reagent grade water, 10mM TRIS-HCL pH 8.0, or TE Buffer (Low TE, <0.1mM EDTA)
- Magnetic separation device compatible with 96 or 384 well PCR plate (see page 5)
- 96-well PCR plate or 384-well PCR plate
- Multichannel pipette

HighPrep PCR - DX: PCR cleanup reaction using a 96-well plate

⚠ Bring the **HighPrep PCR - DX** to room temperature for at least 30 min before use.

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

2. Transfer PCR reaction to an appropriate 96-well plate. For a PCR reaction volume of less than 50 µL, adjust the volume to 50 µL using sterile water.

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

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

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

4. Mix the **HighPrep PCR - DX** 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 ~ 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 40 µL of elution buffer (reagent grade water, TRIS-HCl pH 8.0 or TE buffer) to each well and pipette 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 subsequent applications or store at -20°C for later use.

HighPrep PCR - DX: PCR cleanup reaction using a 384-well plate

⚠ Bring the **HighPrep PCR - DX** to room temperature for at least 30 min before use.

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

2. Transfer PCR reaction to an appropriate 384-well plate. Follow the table in step 3 for the desired sample processing volume.

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

PCR Reaction Volume (µL)	HighPrep PCR - DX Volume at 1.8X (µL)*
5	9
7	12.6

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

4. Mix the **HighPrep PCR - DX** 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 pipette 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 subsequent applications or store at -20°C for later use.

Custom DNA Size-selection Protocols for NGS Applications

To obtain a custom protocol for DNA size-selection of a specific fragment size, contact:

US/Canada: support@magbiogenomics.com

Europe: info.europe@magbiogenomics.com

Ordering

HighPrep PCR - DX

Catalog No.	Product
AC-60005E	HighPrep PCR - DX (5 mL)
AC-60050E	HighPrep PCR - DX (50 mL)
AC-60250E	HighPrep PCR - DX (250 mL)
AC-60500E	HighPrep PCR - DX (500 mL)

Related Products

MagQuant Plus DNA Kit V2 - DX

Catalog No.	Product	Description	Preps
MQP-50096E	MagQuant Plus DNA Kit V2 - DX (96 Preps)	Magnetic bead based kit for normalization of DNA concentration, and quantitation of DNA for NGS and other applications.	96
MQP-50384E	MagQuant Plus DNA Kit V2 - DX (384 Preps)		384
MQP-51920E	MagQuant Plus DNA Kit V2 - DX (1920 Preps)		1920

HighPrep Blood & Tissue DNA Kit - DX

Catalog No.	Product	Description	Preps
HPBTS-D96E	HighPrep Blood & Tissue DNA Kit - DX (96 preps)	Magnetic bead-based kit for Genomic DNA isolation from 20-250 µL of blood, lysate of tissues, mouse tails, cultured cells, or buccal swabs, or blood spot cards.	96
HPBTS-D96x4E	HighPrep Blood & Tissue DNA Kit - DX (384 preps)		384

Magnetic Separation Devices

Catalog No.	Description
MYMAG-96X	Magnetic Separation Device (96 well ring format)
MBMS-12	MagStrip Magnet Stand (1.5 mL x 12)
MBMS-31550	15 mL and 50 mL Magnetic Stand Combo (3 x 15 mL and 3 x 50 mL)

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