AxyPrep™ Mag PCR Normalizer Protocol

(PCR Normalization and Clean-up System)

Summary

AxyPrep Mag PCR Normalizer utilizes a paramagnetic bead-based purification system for PCR DNA, plasmid and genomic DNA normalization. Owing to the limited binding surface of the paramagnetic beads, a pre-defined amount of DNA can be isolated regardless of the DNA input.

The use of the AxyPrep Mag PCR Normalizer helps eliminate the additional steps needed to achieve optimal DNA concentrations amongst multiple samples thereby reducing the amount of time, labor and reagent cost associated with these steps.

The protocol is comprised of three steps, a binding, washing and elution step, that can be performed directly in a 96 well microplate. No centrifugation or filtration steps are required allowing the process to be adaptable to multiple commercially available automation platforms.

Process Overview

1. Bind DNA (e.g. PCR products, plasmid DNA, purified genomic DNA or unpurified PCR reactions and plasmid lysate) to the magnetic beads and then using a magnet, separate the beads from the solution.
2. Wash the beads to remove contaminants such as excess DNA, nucleotides, and salts.
3. Elute DNA

Kit Specifications

The amount of AxyPrep Mag PCR Normalizer used per purification reaction depends on the DNA input. The binding capacity of the AxyPrep Mag PCR Normalizer beads is 200 ng of DNA for 10 ul beads.

Recommendation:
To minimize the variability within each data point, the input DNA must be at least 3-4 times higher than the desired DNA amount. Example: If your desired elution DNA concentration is 2ng/µl, then your sample input must be at least 8ng/µl.

AxyPrep Mag PCR Normalizer Kit Offering

Product Number	Description	Number of Reactions
MAG-PCR-NM-5	AxyPrep Mag PCR Normalizer Kit- 96 preps	96
MAG-PCR-NM-50	AxyPrep Mag PCR Normalizer Kit- 384 preps	384
MAG-PCR-NM-250	AxyPrep Mag PCR Normalizer Kit- 1920 preps	1920

Materials Supplied in the Kit

- **AxyPrep Mag PCR Normalizer Binding Beads**
  - ✓ Store at 2-8°C upon arrival, for up to 16 months.
  - ✓ Before using completely re-suspend the PCR Normalizer paramagnetic beads. The solution should appear homogenous.
  - ✓ For best results the beads should be at **room temperature** prior to use.
  - ✓ DO NOT FREEZE.

- **BB (Binding Buffer); EB-N (Elution Buffer)**
  - ✓ Store at room temperature for up to 16 months.

Equipment and Reagents to Be Supplied by User:

• 70% Ethanol
• 100% Ethanol

Consumables & Hardware to be supplied by the User:

Name	Recommended Model	Recommended Vendor and P/N
96-well PCR reaction plate	96-well round/ flat bottom microtiter plate. Plate selection depends on the PCR reaction volume	Corning, Inc., www.corning.com # 3797, 96 well round bottom # 3591, 96 well flat bottom # 3957, 0.5 mL v bottom 96 # 3365, 360 µL round 96 # 3364, 360 µL flat 96 # 3371, 96 clear pro
	96-well cycling plate	Axygen, PCR-96-FS-C, PCR-96M2-HS-C, www.axygen.com
384-well PCR reaction plate	384 well cycling plate	Axygen, PCR-384M2-C, www.axygen.com
PCR Plate Seals	Easy Peel Heat Sealing Foil	Axygen, MF-111, www.axygen.com
Liquid handling robotics	Compatible with open platform robotics	Contact Axygen Biosciences Technical support for compatible AxyPrep Mag methods and accessories to your automation
Multichannel hand pipette	AxyPet	Single, 8 and 12 Multichannel

IMAG™ Handheld Magnetic Separation Devices Selection Guide:

The IMAG™ handheld Magnetic devices have been designed and optimized for different AxyPrep Mag protocols. These Magnets address different volumes for the tubes and plate types shown below.

Tube based:

Protocol	Manufacturer	Part number	Plate description	Plate Material	Part Number
AxyPrep Mag Kits	Axygen	SCT-050-SS-C	0.5 ml Self Standing Screw cap tube	Polypropylene	**IMAG-12T**
	Axygen	SCT-150-SS-C	1.5 ml Self Standing Screw cap tube	Polypropylene	
	Axygen	SCT-200-SS-C	2.0 ml Self Standing Screw cap tube	Polypropylene	

Plate based:

Protocol	Manufacturer	Part number	Plate description	Plate Material	Part Number
AxyPrep Mag Kits	Corning	3364	96 flat 360ul	Polypropylene	**IMAG-96P**
	Corning	3591	96 flat bottom	Polystyrene	
	Corning	3365	96 round 360ul	Polypropylene	
	Corning	3371	96 clear pro round	Polypropylene	
	Corning	3797	96 round bottom	Polystyrene	
	Corning	3957	96 v bottom 0.5mL	Polypropylene	
	Axygen	PCR-96-FS-C	96 PCR full skirt	Polypropylene	
	Axygen	PCR-96M2-HS-C	96 PCR half skirt		
	Corning	3959	96 round bottom 1ml		
	Corning	3961	96 round bottom 2ml		

AxyPrep Mag PCR Normalizer – Purified DNA Sample (gDNA, amplicons, plasmid DNA)

Procedure in a 96 Well or tube Format:

1. Bring the AxyPrep MAG PCR Normalizer binding beads to room temperature and briefly vortex to fully re-suspend the magnetic beads before using. The reagent should appear homogenous and consistent in color.

2. To isolate 200 ng DNA, add 10 µL of beads to 50 µL of the DNA sample into a magnet compatible 96 well microplate. Mix well by pipetting five times. For a plate to be 'magnet compatible', the bottom of each well should directly contact each magnet.

3. Add 100 µl BB Buffer to the sample well; pipet mix 5 times and incubate the mixture for 10 minutes at room temperature.

4. Place the sample plate on the 96 magnetic separation device for 4 minutes or until the solution clears; with the sample plate still on the magnet, remove and discard the supernatant by pipetting.

5. Remove the sample plate from the magnetic separation device.

6. Add 150 µl freshly prepared 70% ethanol to each well and pipet mix 5 times and incubate for 2 minutes at room temperature.

7. Place the sample plate on the 96 magnetic separation device for 4 minutes or until the solution clears.

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

9. Dry the beads by incubating the plate for 2 minutes at room temperature with the plate still on the magnetic separation device.

10. Remove the sample plate from the magnetic separation device. Add 50µl of EB-N Elution Buffer to each well and pipet up and down 5 times to mix.

11. Incubate the sample plate for 2 minutes at room temperature.
12. Place the sample plate back on the magnetic separation device and wait 4 minute or until the magnetic beads clear from solution.

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

Please contact Axygen Biosciences for sales support at: axgsales@corning.com and for technical support at: axgsupport@corning.com