Axygen® AxyPrep MAG PCR Normalizer Kit

Protocol

CORNING

The Axygen AxyPrep MAG PCR Normalizer Kit is based on paramagnetic beads technology designed to standardize varying DNA concentration without the need for fluorescent measurement or other adjustments to obtain the desired uniform DNA concentration. The DNA being normalized may be from samples of the same source or different sources, however the output remains the same. The Axygen AxyPrep MAG PCR Normalizer Kit is based on the binding of DNA to a proprietary paramagnetic bead with limited binding sites; and excess DNA is washed off, and normalized amounts of DNA are eluted. This protocol does not require a centrifugation step, and can be used in a manual procedure, and the kit is also amenable to automatic liquid handling instruments.

Safety Information

Always wear protective clothing including suitable lab coat, disposable gloves, and protective googles when working with chemicals. Please consult the appropriate material safety data sheets (MSDS) for the product.

Process

The Axygen AxyPrep MAG PCR Normalizer Kit uses a simple 3-step process (bind, wash, elute) that allows the user to obtain equal amounts of DNA regardless of DNA input. Thus, similar sized PCR DNA fragments (purified), plasmid DNA, as well as DNA from PCR reactions (amplicons) and plasmid lysates can be normalized for various downstream applications such as library preparation, NGS, or any other molecular application.

Kit Storage and Stability

The Axygen AxyPrep MAG PCR Normalizer Kit components are stable for at least 12 months from the date of purchase when stored as follows: MAG PCR Normalizer Binding Beads should be stored at 2°C to 8°C. The rest of the kit components should be stored at room temperature (15°C to 25°C). Check buffers for precipitates before use and redissolve any precipitates by warming to 37°C if needed.

Kit Contents and Storage

	Axygen AxyPrep MAG PCR Normalizer Kit Cat. No.				
	MAG-PCR-NM-1	MAG-PCR-NM-5	MAG-PCR-NM-50	MAG-PCR-NM-250	
	10 Preps	96 Preps	384 Preps	1920 Preps	Storage Temperature
MAG PCR Normalizer Binding Beads	110 µL	1 mL	4 mL	20 mL	2°C to 8°C
Binding Buffer (BB)	675 µL	4.5 mL	18 mL	90 mL	15°C to 25°C
Elution Buffer (EB-N)	1.5 mL	10 mL	40 mL	200 mL	15°C to 25°C

Preparation of Reagents

Prepare the following components for each kit before use. **NOTE:** Ensure the bottle/tube lid Is closed tightly when preparing and storing reagents.

Cat. No.	Component	Add 100% Isopropanol	Storage
MAG-PCR-NM-1	Binding Buffer	75 µL	15°C to 25°C
MAG-PCR-NM-5	Binding Buffer	500 µL	15°C to 25°C
MAG-PCR-NM-50	Binding Buffer	2 mL	15°C to 25°C
MAG-PCR-NM-250	Binding Buffer	10 mL	15°C to 25°C

Specifications and Recommendations

The capacity of paramagnetic beads in binding DNA varies based on the size of the DNA fragment and the source of DNA. The amount of DNA that will bind to paramagnetic beads depends on various factors such as extraction protocol efficiency, DNA size, quality, and quantity of input DNA material.

Recommended DNA Input

The recommended minimum DNA input for genomic DNA normalization is 800 ng and maximum input is 2000 ng. For amplicon normalization it is recommend that amplicons should be purified before performing DNA normalization. Unpurified amplicon normalization produces inconsistent results. The minimum input DNA for amplicon normalization is 300 ng and the maximum input is 2000 ng.

1.0 Protocol for Genomic DNA and Amplicon Normalization

NOTE: Bring MAG PCR Normalizer Binding Beads to room temperature for at least 30 min. before use.

1. Pipet 50 µL of genomic DNA or purified PCR product to a 96-well microplate.

   NOTE: For DNA amount less than 50 µL adjust the DNA volume to 50 µL with Elution Buffer or nuclease-free water.

2. Add 50 µL Binding Buffer and 10 µL of MAG PCR Normalizer Binding Beads (shake or vortex MAG PCR Normalizer Binding Beads thoroughly to resuspend the magnetic beads). Mix the DNA sample with Binding Buffer and the beads thoroughly by pipetting or vortexing. Incubate at room temperature for 5 min.
3. Remove the supernatant and discard. Pipet carefully to discard the supernatant, and do not disturb the MAG PCR Normalizer Binding Beads.
4. Leave the sample plate on the magnetic separation device and pipet 150 µL of 80% Ethanol to each well. Incubate for 1 min. at room temperature.
5. With the sample plate still on the magnetic separation device, pipet and discard the cleared supernatant. Do not disturb the MAG PCR Normalizer Binding Beads while discarding the supernatant.
6. Repeat Steps 4 and 5 for a second 80% Ethanol wash step.
7. Leave the sample plate on the magnetic separation device for 5 minutes to air dry. Remove any residual liquid with a pipet without disturbing the MAG PCR Normalizer Binding Beads.

   NOTE: It is important to completely remove all traces of alcohol, but be cautious not to over dry the beads as this will reduce DNA yield.

8. Remove the sample plate from the magnetic separation device. Pipet 25 to 50 µL of Elution Buffer to each sample, and mix thoroughly to resuspend the beads.
9. Seal the plate, and incubate for 5 min. at 65°C.
10. Put the plate on the magnetic separation device to magnetize the MAG PCR Normalizer Binding Beads. The plate should sit at room temperature until the MAG PCR Normalizer Binding Beads are completely cleared from the solution.
11. Transfer the supernatant containing the normalized DNA to a new plate.
12. Store the DNA at -20°C.

2.0 Protocol for PCR Product (Amplicons)

NOTE: Bring MAG PCR Normalizer Binding Beads to room temperature for at least 30 min. before use.

1. To 25 µL of purified PCR, add 25 µL of Binding Buffer and 10 µL of MAG PCR Normalizer Binding Beads (shake or vortex MAG PCR Normalizer Binding Beads thoroughly to fully resuspend the magnetic beads before pipetting). Mix the DNA sample with Binding Buffer by pipetting or vortexing. Incubate at room temperature for 10 min.

   NOTE: If the amplicon amount is less than 25 µL, adjust the volume to 25 µL with Elution Buffer or nuclease-free water.

2. Put the 96-well microplate on the magnetic separation device to magnetize the MAG PCR Normalizer Binding Beads. Allow the MAG PCR Normalizer Binding Beads to magnetize completely until the solution is clear.
3. Remove and discard the supernatant. Make sure not to disturb the MAG PCR Normalizer Binding Beads while discarding the supernatant.
4. Leave the sample plate on the magnetic separation device and pipet 150 µL of 80% Ethanol to each well. Incubate for 1 min. at room temperature.
5. With the sample plate still on the magnetic separation device, pipet and discard the cleared supernatant. Do not disturb the MAG PCR Normalizer Binding Beads while discarding the supernatant.
6. Repeat Steps 4 and 5 for a second 80% Ethanol wash step.
7. Leave the sample plate on the magnetic separation device for 5 min. to air dry. Remove any remaining liquid with a pipet without disturbing the MAG PCR Normalizer Binding Beads.

   NOTE: It is important to completely remove all traces of alcohol, but be cautious not to over dry the beads as this will reduce DNA yield.

8. Remove the sample plate from the magnetic separation device. Pipet 25 to 50 µL of Elution Buffer to each sample, and mix thoroughly to resuspend the beads.
9. Seal the plate, and incubate for 5 min. at 65°C.
10. Place the plate on the magnetic separation device to magnetize the MAG PCR Normalizer Binding Beads. Let the plate sit at room temperature until the MAG PCR Normalizer Binding Beads are completely cleared from solution.
11. Transfer the supernatant containing the normalized amplicons to a new plate.
12. Store the amplicons at -20°C.

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