

Axygen® AxyPrep MAG PCR Clean-up Kit Protocol



The Axygen AxyPrep MAG PCR Clean-up kit utilizes a unique paramagnetic bead technology for rapid, high-throughput purification of PCR amplicons. Using this kit, PCR amplicons 100 base pairs (bp) and larger are selectively bound to paramagnetic beads due to an optimized buffer system. The protocol comprises binding, washing and eluting steps, where primers, nucleotides, salts and enzymes in the reaction mixture are efficiently removed leaving a purified PCR product that is essentially free of contaminants. Furthermore, primer dimers can be removed by using a reduced concentration of AxyPrep MAG PCR Clean-up beads (supplemental protocol).

Product Highlights

- Removes <100 bp product and contaminants
- Automation compatible
- Streamline manual throughput with Axygen IMAG™ handheld magnetic separation device
- No centrifugation or filtration steps
- Simple process completed in 15 minutes Easily scalable to suit multiple applications

Application Areas

- PCR
- Next Generation Fragment Analysis Sequencing
- Genotyping and SNP detection Restriction Enzyme clean up
- Primer walking

The AxyPrep MAG PCR Clean-up process can be performed in a tube, 96-well format using IMAG handheld magnetic separation device, or 384-well format, using a robotic platform.

How it Works

1. To allow DNA binding to the magnetic beads, add 18 µL of AxyPrep MAG PCR clean-up bead solution (1:1.8 ratio) to 10 µL of PCR product. Mix well.
2. Separate beads and DNA from unbound PCR contaminants in the supernatant.
3. Wash beads twice with 70% ethanol to wash away the supernatant.
4. Elute purified PCR products from magnetic beads to a new microplate or tube.

Axygen AxyPrep MAG PCR Clean-up Kits

Cat. No.	Description
MAG-PCR-CL-5	Small, 5 mL
MAG-PCR-CL-50	Medium, 50 mL
MAG-PCR-CL-250	Large, 250 mL
MAG-PCR-CL-500	Bulk, 500 mL

Kits include: Axygen AxyPrep MAG PCR Clean-up paramagnetic bead solution.

- Store at 4°C upon arrival (DO NOT FREEZE), for up to 18 months.
- Prior to each use, bring the reagent to **room temperature** and completely re-suspend the beads through bottle shaking and vigorous vortexing.
- Visually, the solution should be homogenous throughout.

Table 1 illustrates the number of PCR reactions an AxyPrep MAG PCR Clean-up kit can purify depending on the PCR reaction volume. Typical reaction volume is 10-20 µL for the 96-well microplate and 5-10 µL for the 384-well microplate. Number of preps is based on 10 µL reaction volume.

Table 1. Typical reactions per kit based on 10 µL reaction volume.

PCR Reaction Volume (96-well, µL)	No. of Reactions			
	MAG-PCR-CL-5	MAG-PCR-CL-50	MAG-PCR-CL-250	MAG-PCR-CL-500
10	305	3,055	15,275	30,550
20	152	1,527	7,635	15,270
50	61	611	3,055	6,110

Materials Supplied by the User

Consumables and Hardware	Recommended Model	Recommended Vendor (Cat. No.)
96-well PCR reaction microplate	96-well round, flat bottom microplate. Plate selection depends on the PCR reaction volume.	Corning (6509, 6511)
	96-well cycling microplate	Corning (PCR-96-FS-C, PCR-96M2-HS-C)
384-well PCR reaction microplate	384-well cycling microplate	Corning (PCR-384M2-C)
PCR plate seals	Easy peel heat sealing foil	Corning (MF-111)
Liquid handling robotics	Compatible with open platform robotics	Corning (PCR-384-FS-B-C, PCR-96-BRF-B-C)
Multichannel pipettor	Axygen® AxyPet pipettor	Corning (Single-, 8- and 12-multichannel pipettor)

Reagents	Application Step
70% Ethanol	Washing solvent, should be prepared fresh each time
10 mM TRIS-HCl, pH=8.0	DNA elution
Reagent grade water	
10 mM Tris-HCl pH 8.0, 1 mM EDTA	

Axygen IMAG™ Handheld Magnetic Separation Devices

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	Cat. No.	Tube Description	Tube Material	IMAG Cat. No.
AxyPrep MAG Kits	SCT-050-SS-C	0.5 mL self-standing screw cap tube	Polypropylene	IMAG-12T
	SCT-150-SS-C	1.5 mL self-standing screw cap	Polypropylene	
	SCT-200-SS-C	2.0 mL self-standing screw cap	Polypropylene	



Plate-based				
Protocol	Cat. No.	Microplate description	Microplate Material	IMAG Cat. No.
AxyPrep MAG Kits	3364	96-well flat 360 µL	Polypropylene	IMAG-96P
	3591	96-well flat bottom	Polystyrene	
	3365	96-well round 360 µL	Polypropylene	
	3797	96-well round bottom	Polystyrene	
	3957	96-well V-bottom	Polypropylene	
	PCR-96-FS-C	96-well PCR full skirt		
	PCR-96M2-HS-C	96-well PCR half skirt	Polypropylene	
	3959	96-well round bottom 1 mL		
	3961	96-well round bottom 2 mL		

Protocol for 96-well Format

1. Preparation

- a) Determine if a plate transfer is required.

The sample will need to be transferred if the PCR reaction volume, multiplied by 2.8, exceeds the working volume of the PCR plate used. If a transfer is necessary, a 300 µL round bottom microplate is recommended.

- b) Ensure the beads are at room temperature before use and gently shake the AxyPrep MAG PCR Clean-up bottle to re-suspend any magnetic particles that may have settled.

2. Binding

- a) Add AxyPrep MAG PCR Clean-up according to the PCR volume table below:

PCR Volume (µL)	AxyPrep MAG PCR Clean-up Volume at 1.8 x (µL)
10	18
20	36
50	90

NOTE: For a given reaction, the volume of AxyPrep MAG PCR Clean-up can be determined from the following equation:
(Volume of AxyPrep MAG PCR Clean-up per reaction) = 1.8 x (PCR Volume)

- b) Mix PCR reaction solution and the bead reagent thoroughly by pipette mixing 5-10 times and allow the mixture to incubate at room temperature for 5 minutes.

This step allows the binding of PCR products 100 bp and greater to the magnetic beads.

After mixing, the color of the mixture should appear homogenous.

- c) Place the reaction plate onto a 96-well magnet plate or IMAG 96-P for 3 minutes or until the solution clears.

NOTE: It is important to wait until the solution is clear before proceeding to the washing step, otherwise beads may be lost.

- d) While the reaction plate is still on the magnet, aspirate the cleared supernatant and discard.

NOTE: Be careful while aspirating so as not to disturb the settled magnetic beads. If the beads are being drawn into the tips, leave behind a few microliters of the supernatant.

3. Washing

While the reaction plate is still on the magnet, dispense 200 µL of 70% ethanol to each well. Incubate for 30 seconds at room temperature, and aspirate off the ethanol. Repeat for a total of two washes.

NOTE: Disturbing the settled magnetic beads may result in loss of PCR product. It is important to remove as much of the ethanol as possible because residual ethanol may interfere with downstream applications (e.g., subsequent PCR and electrophoresis).

4. Drying

Allowing the plate to stand for 5 minutes at room temperature will help ensure that any residual ethanol has evaporated.

NOTE: Be careful not to allow the beads to dry out completely (the layer of settled beads will appear cracked if this happens) as this will significantly reduce elution efficiency.

5. Elution

- a) Once dry, remove the reaction plate from the magnetic plate and add 40 µL of elution buffer (reagent grade water, TRIS-HCl pH 8.0, or 10 mM Tris-HCl pH 8.0, 1 mM EDTA) to each well. Pipette mix 5 times.

The DNA is rapidly eluted off the magnetic beads and it is not necessary for the beads to go back into the solution for elution to occur. More than 40 µL of elution buffer can be used if a larger end volume is required, but the final concentration will be more dilute. Less than 40 µL will require extra mixing to ensure the liquid comes into contact with the beads and this volume may not be sufficient to elute the entire PCR product. Elution volume can be adjusted based on the desired concentration. The lowest elution volume that can be used is <15 µL.

- To separate the paramagnetic beads from the supernatant, place the reaction plate onto a 96-well magnetic microplate or IMAG for 1 minute or until solution becomes clear. The supernatant now contains the eluted DNA. The beads may be discarded.
- Transfer the eluate supernatant to a fresh plate for storage and analysis.

Protocol for 384-well Format

1. Preparation

- a) Ensure the beads are at room temperature before use and gently shake the AxyPrep MAG PCR Clean-up bottle to re-suspend any magnetic particles that may have settled.

2. Binding

- a) Add the AxyPrep MAG PCR Clean-up beads from Step 1A), according to the following PCR volume table:

PCR Volume (μL)	AxyPrep MAG PCR Clean-up Volume (μL)
5	9
7	12.6
10	18
14	25

NOTE 1: For a given reaction, the volume of AxyPrep MAG PCR Clean-up can be determined from the following equation:
(Volume of diluted AxyPrep MAG PCR Clean-up per reaction) = 1.8 x (PCR Volume)

NOTE 2: Taking into account the maximum volume capacity of a 384-well plate, it is not possible to purify PCR reactions larger than 14 μL. A total volume greater than 39 μL (PCR sample, 14 μL and AxyPrep MAG PCR Clean-up, 25 μL) may overflow the well causing contamination of adjacent wells.

- b) Mix the PCR reaction solution and the bead reagent thoroughly by pipette mixing 15 times and allow the mixture to incubate at room temperature for 5 minutes.

This step allows the binding of PCR fragments >100 bp to the magnetic beads. After mixing, the color of the mixture should appear homogenous.

- c) Place the reaction plate onto a 384-well magnetic plate for 1 minute or until the solution becomes clear.

NOTE: It is important to wait until the solution is clear before proceeding to the washing step, otherwise beads and product may be lost.

- d) While the reaction plate is still on the 384-well magnetic plate aspirate the cleared supernatant from the reaction plate and discard.

The beads will have formed a spot on the side of the well; therefore, it is important to be careful while aspirating so as not to disturb them or PCR product may be lost.

3. Washing

While the reaction plate is still on the magnet, dispense 30 μL of 70% ethanol to each well. Incubate for 30 seconds at room temperature, and aspirate off the ethanol. Repeat for a total of two washes.

NOTE: Disturbing the settled magnetic beads may result in loss of PCR product. It is important to remove as much of the ethanol as possible because residual ethanol may interfere with downstream applications (e.g., subsequent PCR and electrophoresis).

4. Drying

Allowing the plate to stand for at least 1 minute at room temperature will help ensure that any residual ethanol has evaporated. Be careful not to allow the beads to dry out completely (the layer of settled beads will appear cracked if this happens) as this will significantly reduce elution efficiency.

5. Elution

- a) Once dry, remove the reaction plate from the magnetic plate and add 30 μL of elution buffer (reagent grade water, TRIS-HCl pH 8.0, or 10 mM Tris-HCl pH 8.0, 1 mM EDTA) to each well. Pipette mix 5 times.

The DNA is rapidly eluted off the magnetic beads and it is not necessary for the beads to go back into solution for elution to occur. Thirty (30) μL of elution buffer will be sufficient to fully cover the beads. If desired a volume less than 30 μL may be used, but the volume may not be sufficient to elute the entire PCR product and extra mixing will be required to ensure that the liquid comes into contact with all of the beads. Elution buffer volume can be adjusted based on the desired concentration. For maximum recovery, minimum elution volume is 25 μL.

- b) To separate the paramagnetic beads from the supernatant place the reaction plate onto a 384-well magnetic plate for 1 minute or until solution becomes clear. The supernatant now contains the eluted DNA. The beads may be discarded.
- c) Transfer the eluate supernatant to a fresh plate for storage and analysis.

NOTE: If there is some bead carry over to the new plate the beads will not interfere with subsequent thermal cycling reactions.

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