AxyPrep™ Mag PCR Clean-up Protocol

Intro

The 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 60 base pairs (bp) and larger are selectively bound to paramagnetic beads due to an optimized buffer system. The protocol comprises of 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	Application Areas
Removes <60 bp product and contaminants	PCR
Automation compatible	Sequencing: Sanger and Next Generation
Streamline manual throughput with IMAG™	Fragment Analysis
No centrifugation or filtration steps	Genotyping and SNP detection
Simple process completed in 15 minutes	Restriction Enzyme clean up
Easily scalable to suit multiple applications	Primer walking

How it works

1. To 10 µl of PCR product, add 18 µL of AxyPrep Mag PCR clean-up bead solution (1:1.8 ratio) to allow DNA binding to the Magnetic beads. 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 plate or tube.

AxyPrep Mag PCR Clean-up Kits

AxyPrep Mag PCR Clean-up Products	Part Number
AxyPrep Mag PCR Clean-up - Small 5 mL	Mag-PCR-CL-5
AxyPrep Mag PCR Clean-up - Medium 50 mL	Mag-PCR-CL-50
AxyPrep Mag PCR Clean-up - Large 250 mL	Mag-PCR-CL-250

The AxyPrep Mag PCR Clean-up process can be performed in a tube, 96 well format using IMAG™ or 384 well format, using a robotic platform.

The following table 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.

Typical reactions per kit based on 10 µl reaction volume

PCR Reaction Volume (96 well, µL)	MAG-PCR-CL-5 (# reactions)	MAG-PCR-CL-50 (# reactions)	MAG-PCR-CL-250 (# reactions)
10	305	3055	15275
20	152	1527	7635
50	61	611	3055

Materials Supplied in the Kit

AxyPrep Mag PCR Clean-up paramagnetic bead Solution

- ✓ Store at 4°C upon arrival (DO NOT FREEZE), for up to 12 months
- ✓ Bring the reagent to **room temperature** and to completely re-suspend the beads prior to each use.
- ✓ Visually, the solution should be homogenous throughout.

Materials to be supplied by the User:

Consumables & Hardware:

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 for automation
Multichannel hand pipette	AxyPet	Single, 8 and 12 Multichannel

Reagents:

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	

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 360 µL	Polypropylene	**IMAG-96P**
	Corning	3591	96 flat bottom	Polystyrene	
	Corning	3365	96 round 360 µL	Polypropylene	
	Corning	3371	96 clear pro round	Polypropylene	
	Corning	3797	96 round bottom	Polystyrene	
	Corning	3957	96 v bottom 0.5 mL	Polypropylene	

	Axygen Axygen Corning Corning	PCR-96-FS-C PCR-96M2-HS-C 3959 3961	96 PCR full skirt 96 PCR half skirt 96 round bottom 1 mL 96 round bottom 2 mL	Polypropylene	

Procedure in 96 Well Format:

1. PREPARATION STEP:

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 plate 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.

3. BINDING STEP:

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

PCR Volume (µL)	**AxyPrep Mag PCR Clean-up Volume at 1.8X** (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 times and allow the mixture to incubate at room temperature for 5 minutes.

This step allows the binding of PCR products 125 bp and greater to the Magnetic beads. Please refer to supplemental protocol on page 8 to isolate PCR products between 60 and 125 bp.

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.

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 magnet aspirate the cleared supernatant and discard.

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.

4. WASH STEP:

a) While the reaction plate is still on 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.

Disturbing the settled magnetic beads may result in lose 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).

NOTE: Allowing the plate to stand for 5 minutes 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 STEP:

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 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.

b) To separate the paramagnetic beads from the supernatant place the reaction plate onto a 96 well magnetic plate or IMAG™ 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.

Protocol for the 384 Well Format:

1. PREPARATION STEP:

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 STEP:

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 products 125 bp and greater to the Magnetic beads. After mixing, the color of the mixture should appear homogenous.

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

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 STEPS:

a) While the reaction plate is still on 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.

Disturbing the settled magnetic beads may result in lose 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).

NOTE: 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.

4. ELUTION STEP:

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. 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.

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.

SUPPLEMENTAL PROTOCOL

Recovery of DNA smaller than 125 bp

For optimal recovery of DNA products smaller than 125 bp either add:

i) Isopropanol to the **AxyPrep Mag PCR Clean-up** reagent prior to use.

- or -

ii) Isopropanol to the bead-sample mix prior to applying the magnet.

1. **Add isopropanol to beads prior to starting the purification process**.

   Mix 70 µL of 100% Isopropanol with 180 µL of **AxyPrep Mag PCR Clean-up** bead solution to yield a final concentration of 28% isopropanol.

   For every 10 µL of PCR product, add 25 µL of bead-isopropanol mix (1:2.5 ratio (product:beads)) .

   Note. If using automation with the diluted AxyPrep Mag PCR Clean-up reagent, it may be easier to set the PCR sample volume on the robot to 1.4 fold of the actual volume. For instance, if there is 10 µL of the PCR product; enter 14 µL into the system so that 25 µL of the diluted AxyPrep Mag PCR reagent will be added instead of the typical 18 µL.

2. **Add 100 % isopropanol to the sample-bead mix to yield a final concentration of 20 %. For example, add 7 µL of 100 % isopropanol to 28 µL of bead/sample mix (18 µL beads, 10 µL sample).**

Removal of primer dimer or DNA smaller than 200 bp

If longer primer sets are to be used and primer dimer contamination occurs, it is possible to remove the primer dimers by using diluted **AxyPrep Mag PCR Clean-up** reagents.

1. Perform a sequential titration to determine the best dilution of the AxyPrep Mag PCR clean-up reagent for the PCR product.

a) Add 2 µL, 4 µL, or 8 µL of water into 18 µL of **AxyPrep Mag PCR Clean-up** reagent and test for primer dimer or DNA removal.

b) Use the optimized dilution for subsequent PCR purification.

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

** The PCR process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffman-La Roche, Ltd.*