AxyPrep™ Mag DyeClean

(Sequencing Dye-Terminator Removal System)

Summary

Removing excess dye terminator is an essential step prior to Sanger sequencing. Carryover of the excess dye into the sequencing reactions may result in dye blobs resulting in inaccurate results. The AxyPrep™ Mag DyeClean kit utilizes a unique paramagnetic bead-based purification system for Sanger sequencing reaction dye terminator clean-up. The protocol is simple and comprises binding, washing and elution steps which, if desired, can be performed directly in the thermal cycling plate. This kit requires no centrifugation or filtration steps making it more amenable to automation.

The kit is suitable for use with various dye terminator chemistries including Big Dye versions 1.1 and 3.1 and DYEnamic ET.

Product Highlights
Simple clean-up process completed in 25 minutes/96 samples
Automation compatible
Streamline manual throughput using IMAG™
No centrifugation or filtration steps required
Easily scalable from tubes, through 96 and 384 well microplates
High signal to noise ratio resulting in long reads and high QV20+ scores

Overview of Process:

- Bind sequencing extension products to magnetic beads, and separate on magnet plate.
- Wash beads to remove unincorporated dyes, nucleotides, salts, and other contaminants.
- Elute DNA using aqueous buffer.

AxyPrep MAG DyeClean Kits

The volume of AxyPrep Mag DyeClean required per sequencing reaction depends on plate format **ONLY.** Please refer to the charts below to determine how many clean-ups each kit can provide.

Reaction volume	**5 mL (MAG-DYCL-5)**	**50 mL (MAG-DYECL-50)**	**250 mL (MAG-DYECL-250)**
96 Well Format	500	5000	25000
384 Well Format	1000	10000	50000

Materials Supplied in the Kit

- ✓ AxyPrep Mag DyeClean beads only

- Store at 4°C upon arrival, for up to 12 months.
- DO NOT FREEZE.

Materials to be provided 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 to your automation
multichannel hand pipette	AxyPet	Single, 8 and 12 Multichannel

Reagents to be supplied by the user:

- ✓ 85% Ethanol made from non-denatured ethanol
  - 25 mL of 85% ethanol per 96 well plate is required
  - For best results it is recommended that the 85% ethanol is prepared no more than 3 days prior to use and stored in a tightly capped container.
- ✓ Elution Buffer: Reagent grade water or 0.1 mM EDTA (pH 8.0) – dependent upon sequencing instrument is available
  - 0.1 mM EDTA (pH 8.0) is used to lower the signal in cases where the signal is too strong for certain sequencing instruments
  - Reagent grade water may be used to provide maximum signal
  - Please refer to Elution step table on page 5

IMAG™ Handheld Magnetic Separation Devices Selection Guide:

(Not provided in the Kit – please contact your Corning Axygen supplier for details on IMAG™)

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	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		

Procedure for 96 Well Format:

1. **PREPARATION STEP:**
   - **a.** Briefly vortex the AxyPrep Mag DyeClean paramagnetic beads to fully re-suspend the beads prior to use. For optimal results, the reagent should be at **room temperature** and appear homogenous and consistent in color before use.

2. **BINDING STEP:**
   - **a.** Add 10 µL of AxyPrep Mag DyeClean to each sample. This is a fixed volume and is independent of the sequencing reaction volume.
   - b. Add freshly prepared, 85% ethanol, to each sample according the table below. Pipette mix 7 times or until the solution is homogenous. It is critical that the solution is homogenous for complete binding of the sequencing products to the magnetic beads.

AxyPrep Mag DyeClean for 96 well plates		
Sequencing Reaction Volume (µL)	**AxyPrep Mag DyeClean Volume (µL)**	**Volume of 85% Ethanol (µL)**
5	10	31
10	10	41

Note: For a given reaction, the volume of 85% Ethanol can be determined from the following equation:

Volume of 85% Ethanol = 2.077 x (AxyPrep Mag DyeClean Volume (10 µL) + Sequencing Reaction Volume)

Observation: If the sample is not well mixed the ethanol will float to the top of the sample mixture, and the AxyPrep Mag DyeClean will sink to the bottom

   - c. Place the sample plate onto a 96 well magnetic plate for 3 – 5 minutes or until solution is clear. The magnetic beads will form a tight pellet on the side of the well if using the IMAG™ or a crescent on the side of the well for other magnetic plates.

3. **WASHING STEP:**

All subsequent washes are performed while the plate is situated on the magnet.

   - **a. Aspirate the cleared solution slowly from the beads and discard.**
     The solution contains excess fluorescent dye and contaminants therefore it is critical to remove as much as possible without disturbing the beads.

   - **b. Add 100 µL of 85% ethanol to each sample. Incubate for at least 30 seconds to allow the beads to resettle before continuing to the next step.**

     It is not necessary to mix or re-suspend the beads during this step.

   - **c. Completely remove the ethanol without disturbing the beads.**

d. **Repeat steps b and c for a total of two 85% ethanol washes.**

e. **Let the samples air-dry for 10 minutes at room temperature.**
The sample plate can be situated on or off the magnet while drying.
Note: Excessive drying can lead to degradation of the fluorescent dye bound to the DNA. The beads will appear cracked if over-dried.

4. **ELUTION STEP:**
   a. To elute remove the plate from the magnet, add 40 µL of elution buffer (see chart below) and incubate the plate for 5 minutes at room temperature. Elution of the sequencing products is rapid therefore it is not necessary for the beads to go back into solution for complete recovery.

Notes: The suggested elution buffers are either reagent grade water or 0.1 mM EDTA (pH 8.0). Selecting the appropriate elution buffer will depend on the multiple variables including sensitivity of the sequencing detector, the amount of BigDye used per sequencing reaction and the type of template to be sequenced. Using water as an elution buffer will typically provide the maximum signal, while EDTA may be used to lower the signal. A lower signal may be desired if the fluorescent dye yields a signal intensity beyond the capabilities of detection for the instrument. Please use the following table as a general guideline for choosing an elution buffer.

	ABI 3100 / 3130	ABI 3700	ABI 3730
>2 µL BigDye with PCR Products	0.1 mM EDTA	0.1 mM EDTA	0.1 mM EDTA
<2 µL BigDye with PCR Products	0.1 mM EDTA	Di H2O	0.1 mM EDTA
>2 µL BigDye with Plasmids	Plasmids 0.1 mM	Di H2O	0.1 mM EDTA
<2 µL BigDye with Plasmids	Plasmids Di H2O	Di H2O	Di H2O

   b. Place the reaction plate back on to the magnet to separate the beads from the eluate. Incubate at room temperature for 3 – 5 minutes or until the solution becomes clear. The sequencing product is now in the eluate.

   c. Transfer 35 µL of the clear eluate into a new plate for loading on the detector.

   d. Leave 5 µL – 10 µL of the solution in the plate to prevent transfer of beads into the detector. Residual beads may interfere with the sequencing instrument function.

Storage: Seal samples and store at 4 °C, for up to 24 hours, prior to loading. If samples will not be loaded within 24 hours, store at -20 °C. Samples are stable at -20 °C for at least 1 month.

Procedure for 384 Well Format:

1. **PREPARATION STEP:**
   - **a.** Briefly vortex the AxyPrep Mag DyeClean paramagnetic beads to fully re-suspend the beads prior to use. For optimal results, the reagent should be at **room temperature** and appear homogenous and consistent in color before use.

2. **BINDING STEP:**
   - **a.** Add 5 µL of AxyPrep Mag DyeClean to each sample. This is a fixed volume and is independent of the sequencing reaction volume.

   b. Add freshly prepared, 85% ethanol, to each sample according the table below. Pipette mix 7 times or until the solution is homogenous. It is critical that the solution is homogenous for complete binding of the sequencing products to the magnetic beads.

AxyPrep Mag DyeClean for 384 well plates		
Sequencing Reaction Volume (µL)	**AxyPrep Mag DyeClean Volume (µL)**	**Volume of 85% ethanol (µL)**
5	5	14.3
10	5	21.4

Note: For a given reaction, the volume of 85% Ethanol can be determined from the following equation:

Volume of 85% Ethanol = 1.428 x (5 µL + Sequencing Reaction Volume)

Observation: If the sample is not well mixed the ethanol will float to the top of the sample mixture, and the AxyPrep Mag DyeClean will sink to the bottom

   c. Place the sample plate onto a magnetic plate for 3 – 5 minutes or until solution is clear. The magnetic beads will form a crescent on the side of the well.

5. **WASHING STEP:**

All subsequent washes are performed while the plate is situated on the magnet.

   - **a. Aspirate the cleared solution slowly from the beads and discard.**
     The solution contains excess fluorescent dye and contaminants therefore it is critical to remove as much as possible without disturbing the beads.

   - **b. Add 30 µL of 85% ethanol to each sample. Incubate for at least 30 seconds to allow the beads to resettle before continuing to the next step.**

     It is not necessary to mix or re-suspend the beads during this step.

   - **c. Completely remove the ethanol without disturbing the beads.**

d. Repeat steps b and c for a total of two 85% ethanol washes.

e. Let the samples air-dry for 10 minutes at room temperature.
The sample plate can be situated on or off the magnet while drying.
Note: Excessive drying can lead to degradation of the fluorescent dye bound to the DNA. The beads will appear cracked if over-dried.

6. **ELUTION STEP:**

a. To elute remove the plate from the magnet, add 15 - 30 µL of elution buffer (see chart below) and incubate the plate for 5 minutes at room temperature. Elution of the sequencing products is rapid therefore it is not necessary for the beads to go back into solution for complete recovery.

Notes: The suggested elution buffers are either reagent grade water or 0.1 mM EDTA (pH 8.0). Selecting the appropriate elution buffer will depend on the multiple variables including sensitivity of the sequencing detector, the amount of BigDye used per sequencing reaction and the type of template to be sequenced. Using water as an elution buffer will typically provide the maximum signal, while EDTA may be used to lower the signal. A lower signal may be desired if the fluorescent dye yields a signal intensity beyond the capabilities of detection for the instrument. Please use the following table as a general guideline for choosing an elution buffer.

	ABI 3100 / 3130	ABI 3700	ABI 3730
>2 µL BigDye with PCR Products	0.1 mM EDTA	0.1 mM EDTA	0.1 mM EDTA
<2 µL BigDye with PCR Products	0.1 mM EDTA	Di H2O	0.1 mM EDTA
>2 µL BigDye with Plasmids	Plasmids 0.1 mM	Di H2O	0.1 mM EDTA
<2 µL BigDye with Plasmids	Plasmids Di H2O	Di H2O	Di H2O

b. Place the reaction plate back on to the magnet to separate the beads from the eluate. Incubate at room temperature for 3 – 5 minutes or until the solution becomes clear. The sequencing product is now in the eluate.

c. Transfer the clear eluate into a new plate for loading on the detector.

d. Leave 2 – 5 µL of the solution in the plate to prevent transfer of beads into the detector. Residual beads may interfere with the sequencing instrument function.

Storage: Seal samples and store at 4 °C, for up to 24 hours, prior to loading. If samples will not be loaded within 24 hours, store at -20 °C. Samples are stable at -20 °C for at least 1 month.

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.*