Protocol

Description

AppMag Dye Terminator Removal Clean Up Beads are based on paramagnetic bead technology designed for use in Sanger Sequencing reactions to remove unincorporated terminators. The protocol involves binding, washing and then eluting steps that allows the efficient removal of nucleotides, primers and non-targeted amplicons. DNA that is selectively bound to the magnetic bead particles is finally eluted leaving purified DNA that can then be used straight away in downstream applications

AppMag PCR Clean Up Beads can be used for a manual procedure as well as being compatible with automatic liquid handling instruments.

Process

The protocol consists of a 3-step procedure: Bind-Wash-Elute.
AppMag PCR Clean Up Beads are combined with the PCR reaction sample. The protocol utilizes a magnet plate (can be magnetic stand or plate depending if using tubes or 96 well plate) for processing the PCR reaction sample. Contaminants and salts are removed and pure DNA is eluted.

Product Specifications

Product Code	Product Description	Number of Reactions	Storage Conditions
AMB003	AppMag Dye Terminator Removal Clean Up Beads 50ml	5,000	4-8°C DO NOT FREEZE
AMB004	AppMag Dye Terminator Removal Clean Up Beads 250ml	25,000	

Number of reactions is based on 10µL reaction volume. A 10µL Volume of AppMag Dye Terminator Removal Clean Up Beads is used regardless of the volume of the sequencing reaction.

Materials supplied in the kit:

AppMag Dye Terminator Removal Clean Up bead solution

- DO NOT FREEZE
- Store at 4°C
- Stable for 12 months at 4°C
- Always re-suspend beads before use. Solution should always appear homogenous throughout before pipetting.

Materials Supplied by User:

85% ethanol (Prepared from non-denatured ethanol – so from absolute ethanol)
Elution buffer (0.1mM EDTA or deionised water
Polypropylene reserv
Magnetic plate or stand
Reaction plate (96/384 well)

Name	Recommended Model	Product and P/N
96-well reaction Plate	96-well cycling plate	https://www.appletonwoods.co.uk/product-category/consumables/pcr-plastics/pcr-plates/
384-well reaction Plate	384-well cycling plate	https://www.appletonwoods.co.uk/product/384-well-pcr-plates-axygen/
Polypropylene Reservoirs	Selection of reservoir volume will depend on step in protocol it is used for (10, 25 or 50ml)	https://www.appletonwoods.co.uk/product/reagent-reservoirs-sterile-appleton/
Liquid Handling Robotics	Compatible with open platform robotic systems	https://www.appletonwoods.co.uk/product-category/consumables/pipettes-and-liquid-handling/pipette-tips/robotic-tips/
Single/Multichannel Pipettes	AppPette pipettors	https://www.appletonwoods.co.uk/product/apppette-single-channel-variable-pipettors-appleton/ https://www.appletonwoods.co.uk/product/apppette-multi-channel-variable-pipettors-appleton/
Filter Tips	Filter Pipette tips, low retention, graduated, Appleton	https://www.appletonwoods.co.uk/product/filter-pipette-tips-low-retention-graduated-appleton/

Protocol 96 Well Format

1. Allow AppMag Dye Terminator Removal Clean Up Beads to come to room temperature for at least 30 min before use and shake thoroughly to re-suspend the beads

2. Add 10 µl of the AppMag Dye Terminator Clean Up beads to each sample regardless of the sequencing reaction volume.

3. Add freshly prepared 85% ethanol from absolute ethanol (and not denatured ethanol) according to the table below

Reaction Volume	85 Ethanol (µl)
5	30
10	40
15	50
20	60

4. Mix the AppMag Dye Terminator Removal Clean Up beads and sample thoroughly by pipetting up and down 8-10 times

5. Place the sample plate on the magnetic separation device for 3-5 minutes or until the beads pull to the side of the well and the solution clears

6. Without disturbing the beads and with the sample plate still on the magnet remove and discard the supernatant using a pipette. Be extremely careful not to disrupt the beads attached to the side of the well.

7. Keeping the plate on the magnet stand, add 100 µl of 85% ethanol to each well and incubate for 1-2 mins or until the magnetic beads have fully resettled. There is no need to mix the samples in each well.

8. Remove and discard the supernatant by pipetting whilst leaving the plate on the magnetic stand.

9. Repeat steps 7-8 to complete two 85% ethanol washes

10. Incubate the plate for 10-15 minutes at room temperature to dry the beads with the plate still on the magnetic separation device. It is critical to completely remove all traces of alcohol since this will contain contaminants and traces of excess fluorescent dye.

11. Remove the sample plate from the magnetic separation device and add 40µl of elution buffer (0.1mM EDTA or deionised water) and pipette up and down 15-20 times to mix.

12. Incubate at room temperature for 5 minutes

13. Place the sample plate back on the magnetic separation device for 4-5 minutes or until the solution clears

14. Transfer 30-35 µl the eluate to a new plate to be loaded onto the sequencer.

Protocol 384 Well Format

1. Allow AppMag Dye Terminator Removal Clean Up Beads to come to room temperature for at least 30 min before use and shake thoroughly to re-suspend the beads

2. Add 5 µl of the AppMag Dye Terminator Clean Up beads to each sample regardless of the sequencing reaction volume into the 384 well plate.

3. Add freshly prepared 85% ethanol from absolute ethanol (and not denatured ethanol) according to the table below

Reaction Volume (5 µl)	85% Ethanol (µl)
5	14.3
10	21.4
15	28.6

4. Mix the AppMag Dye Terminator Removal Clean Up Beads and sample thoroughly by pipetting up and down 8-10 times

5. Place the sample plate on the magnetic separation device for 3-4 minutes or until the beads pull to the side of the well and the solution clears

6. Without disturbing the beads and with the sample plate still on the magnet remove and discard the supernatant using a pipette. Be extremely careful not to disrupt the beads attached to the side of the well.

7. Keeping the plate on the magnet stand, add 30 µl of 85% ethanol to each well and incubate for 1-2 mins or until the magnetic beads have fully resettled. There is no need to mix the samples in each well.

8. Remove and discard the supernatant by pipetting whilst leaving the plate on the magnetic stand.

9. Repeat steps 7-8 to complete two 85% ethanol washes

10. Incubate the plate for 10-15 minutes at room temperature to dry the beads with the plate still on the magnetic separation device. It is critical to completely remove all traces of alcohol since this will contain contaminants and traces of excess fluorescent dye.

11. Remove the sample plate from the magnetic separation device and add 15-20 µl of elution buffer (0.1mM EDTA or deionised water) and pipette up and down 15-20 times to mix.

12. Incubate at room temperature for 5 minutes

13. Place the sample plate back on the magnetic separation device for 4-5 minutes or until the solution clears.

14. Transfer the eluate to a new plate to be loaded onto the sequencer.

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