Protocol

Description

AppMag PCR Clean Up Beads are based on paramagnetic bead technology designed for purification of amplicons 60 base pairs (bp) and larger. The protocol involves binding, washing and then eluting steps that allows the efficient removal of primers, primer-dimers, salts and dNTPs. DNA fragments are selectively bound to the magnetic bead particles leaving purified DNA following elution with low salt elution buffer or water that can then be used straight away in the following applications:

- PCR
- Mutation detection and genotyping
- Sequencing (Sanger and Next Generation)
- Cloning
- Restriction enzyme clean up
- Microarrays

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

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
AMB001	AppMag PCR Clean Up Beads 50ml	2,778	4-8°C DO NOT FREEZE
AMB002	AppMag PCR Clean Up Beads 250ml	13,890	

Number of reactions is based on 10µL PCR reaction volume. Volume of AppMag PCR Clean Up Beads per reaction = 1.8x reaction volume

Materials supplied in the kit:

AppMag PCR clean up bead solution

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

Materials Supplied by User:
80% ethanol (Prepared from non-denatured ethanol)
10mM TRIS-HCL pH 8.0 (DNA Elution)
Reagent grade water
1mM EDTA
Magnetic plate or stand
Reaction plate (96/384 well)

Name	Recommended Model	Product and P/N
96-well PCR reaction Plate	96-well round/flat bottom plate – plate selection depends on PCR reaction volume used	https://www.appletonwoods.co.uk/product-category/consumables/pcr-plastics/pcr-plates/
384-well PCR reaction Plate	384-well cycling plate	https://www.appletonwoods.co.uk/product/384-well-pcr-plates-axygen/
PCR plate seals	Heat Seals	https://www.appletonwoods.co.uk/product-category/consumables/pcr-plastics/heat-seals/
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 PCR Clean Up Beads to come to room temperature for at least 30 min before use and shake thoroughly to re-suspend the beads

2. Transfer the PCR reaction to a 96 well plate. For a 50µl reaction add sterile water to the appropriate adjusted volume

3. Add the correct volume of AppMag PCR Clean Up Beads according to the table below

PCR Reaction Volume (µl)	AppMag PCR Clean Up Beads Volume (µl) (1.8x PCR Reaction Volume)
10	18
20	36
50	90

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

5. Incubate the mixture for 5 minutes at room temperature

6. 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

7. Without disturbing the beads and with the sample plate still on the magnet remove and discard the supernatant using a pipette

8. Keeping the plate on the magnet stand, add 200 µl of 80% ethanol to each well and incubate for 30 seconds at room temperature

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

10. Repeat steps 8-9 for a total of 2 washes of 80% ethanol

11. 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

12. Remove the sample plate from the magnetic separation device and add 40µl of elution buffer (reagent grade water, TRIS-HCl pH 8.0 or TE buffer) and pipette up and down 5-6 times to mix. Warming the elution buffer to 55°C can help increase the yield

13. Incubate at room temperature for 2 minutes

14. Place the sample plate back on the magnetic separation device for 2-3 minutes or until the solution clears

15. Transfer the eluate to a new plate for subsequent downstream applications or for storage

Protocol 384 Well Format

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

2. Transfer the PCR reaction to a 384 well plate. For 50 µl reaction adjust volume appropriately using sterile water

3. Add the correct volume of AppMag PCR Clean up beads according to the table below

PCR Reaction Volume (µl)	AppMag PCR Clean Up Beads Volume (µl) (1.8 x PCR Reaction Volume)
5	9
7	12.6
10	18

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

5. Incubate the mixture for 5 minutes at room temperature

6. Place the sample plate on the magnetic separation device for 1-2 minutes or until the beads pull to the side of the well and the solution clears

7. Without disturbing the beads and with the sample plate still on the magnet remove and discard the supernatant using a pipette

8. Add 30 µl of 80% ethanol to each well and incubate for 30 seconds at room temperature

9. Remove and discard the supernatant by pipetting

10. Repeat steps 8-9 for a total of 2 washes of 80% ethanol

11. Incubate the plate for 4-5 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

12. Remove the sample plate from the magnetic separation device and add 30µl of elution buffer (reagent grade water, TRIS-HCl pH 8.0 or TE buffer) and pipette up and down 5-6 times to mix. Warming the elution buffer to 55°C can help increase the yield

13. Incubate at room temperature for 2 minutes

14. Place the sample plate back on the magnetic separation device for 2-3 minute or until the solution clears.

15. Transfer the eluate to a new plate for subsequent downstream applications or storage

Appleton Woods Ltd
New Lindon House
Catesby Park
Kings Norton
Birmingham
B38 8SE