

## 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<br>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 | <a href="https://www.appletonwoods.co.uk/product-category/consumables/pcr-plastics/pcr-plates/">https://www.appletonwoods.co.uk/product-category/consumables/pcr-plastics/pcr-plates/</a>  |
| 384-well PCR reaction Plate  | 384-well cycling plate  | <a href="https://www.appletonwoods.co.uk/product/384-well-pcr-plates-axygen/">https://www.appletonwoods.co.uk/product/384-well-pcr-plates-axygen/</a>  |
| PCR plate seals              | Heat Seals  | <a href="https://www.appletonwoods.co.uk/product-category/consumables/pcr-plastics/heat-seals/">https://www.appletonwoods.co.uk/product-category/consumables/pcr-plastics/heat-seals/</a>  |
| Liquid Handling Robotics     | Compatible with open platform robotic systems   | <a href="https://www.appletonwoods.co.uk/product-category/consumables/pipettes-and-liquid-handling/pipette-tips/robotic-tips/">https://www.appletonwoods.co.uk/product-category/consumables/pipettes-and-liquid-handling/pipette-tips/robotic-tips/</a>  |
| Single/Multichannel Pipettes | AppPette pipettors  | <a href="https://www.appletonwoods.co.uk/product/apppette-single-channel-variable-pipettors-appleton/">https://www.appletonwoods.co.uk/product/apppette-single-channel-variable-pipettors-appleton/</a><br><a href="https://www.appletonwoods.co.uk/product/apppette-multi-channel-variable-pipettors-appleton/">https://www.appletonwoods.co.uk/product/apppette-multi-channel-variable-pipettors-appleton/</a> |
| Filter Tips                  | Filter Pipette tips, low retention, graduated, Appleton                               | <a href="https://www.appletonwoods.co.uk/product/filter-pipette-tips-low-retention-graduated-appleton/">https://www.appletonwoods.co.uk/product/filter-pipette-tips-low-retention-graduated-appleton/</a>  |

## 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 50 $\mu$ l 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 ( $\mu$ l) | AppMag PCR Clean Up Beads Volume ( $\mu$ l)<br>(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  $\mu$ l 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 40 $\mu$ l 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  $\mu$ l 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 ( $\mu$ l) | AppMag PCR Clean Up Beads Volume ( $\mu$ l)<br>(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  $\mu$ l 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 30 $\mu$ l 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



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