AbraMag® PCR Clean-Up Magnetic Beads are intended for research and *in vitro* use only. This product was not tested or certified for diagnostic use.

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AbraMag® PCR Clean-Up Magnetic Beads

Product No. 555030 (5 mL), **555035** (60 mL), **555040** (450 mL)

1. General Description

AbraMag® PCR Clean-Up Magnetic Beads are designed to purify PCR amplicons > 100bp out of a PCR reaction for use in downstream applications. Amplicons are reversibly bound to paramagnetic particles while excess primers, salts, enzymes, free nucleotides, and other non-targets left in the reaction are washed away. The beads are designed for use with a magnetic separator rack.

2. Safety Instructions

Always use appropriate protective equipment (including but not limited to gloves, lab coats, and safety glasses) when working with nucleic acids. Refer to Safety Data Sheet for further information.

3. Storage and Stability

Upon delivery, store at 4°C. **Do not freeze the magnetic beads solution**. Do not use after the printed expiration date.

4. Principle

The **AbraMag® PCR Clean-Up Magnetic Beads** process uses a simple, efficient, magnetic bead-based procedure for PCR amplicon purification from a PCR reaction, as illustrated below in **Figure 1**:

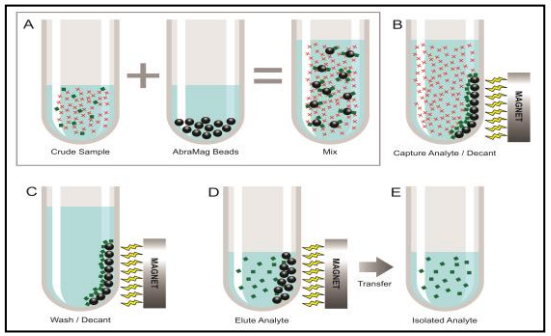

Figure 1. Schematic of the **AbraMag® PCR Clean-Up Magnetic Beads** process.

4.A. **Mixing:** The PCR reaction sample is added to AbraMag® magnetic beads.

4.B. **Binding:** PCR amplicons > 100 bp bind to the beads in the presence of the optimized buffer. A magnet is used to secure the beads.

4.C. **Washing:** Primers and other unwanted reagents are washed away in a series of two wash steps.

4.D. **Elution:** PCR amplicons are then eluted and transferred to a new tube.

4.E. **Downstream Applications:** Pure, high-quality isolated amplicons may then be used for downstream procedures such as PCR, cloning, and sequencing.

5. Limitations and Precautions

PCR clean-up should be performed soon after the PCR reaction is complete, or PCR product should be stored at 4°C until clean-up. If selection of a specific fragment size is desired, use **AbraMag® DNA Size Selection Magnetic Beads** (PN 544100 (5 mL), 544103 (60 mL), 544106 (450 mL)).

6. Working Instructions

6.A. **Materials Provided**

1. AbraMag® PCR Clean-Up Magnetic Beads

6.B. **Additional Materials and Equipment Required** (not included with the product)

1. Amplified PCR product
2. Laboratory-grade water
3. Freshly prepared 70% Ethanol
4. Molecular biology-grade TE buffer pH 8.0 (10 mM Tris, 1 mM EDTA pH 8.0)
5. Disposable gloves and other protective equipment
6. Micro-pipettes with disposable plastic filter barrier tips
7. 1.5 mL sterile, nuclease-free microcentrifuge tubes
8. 4°C refrigerator
9. Magnetic microcentrifuge tube separator, Solo (PN 472270), Multi-6 (PN 472260), Microtiter Plate Side-Pull (PN 472235) or Bottom-Pull (PN 472236),or similar
10. Vortexer

6.C. **Procedure**

1. Transfer the PCR product to a 1.5 mL microcentrifuge tube. Dilute the sample to 50 µL using laboratory-grade water.
   Note: A small amount of PCR product can be saved for analysis. See section 7.

2. Vortex the **AbraMag® PCR Clean-Up Magnetic Beads** with ~1 second pulses. Ensure that the solution is completely resuspended. Add 40 µL of bead solution to the PCR product sample. Pipette up and down to mix well.

3. Incubate the sample/bead mixture for 5 minutes at room temperature.

4. Place the sample on the magnetic separator until the solution is clear (~1 minute). Leaving the tube on the separator, aspirate and discard the supernatant without disturbing the beads that have gathered at the magnet.

5. Leaving the tube on the separator, gently add 200 µL of freshly prepared 70% ethanol, without dislodging the beads from the side of the tube. Let sit for 30 seconds. Aspirate and discard the supernatant.

6. Repeat Step 5 for a second wash. Be careful to remove all pipettable ethanol.

7. Remove the tube from the magnetic separator and leave at room temperature for ~3 minutes with the cap open to completely evaporate any residual ethanol.

8. Add 40 µL TE buffer pH 8.0 or laboratory-grade water to the sample. Pipette up and down to mix well. Incubate at room temperature for 2-3 minutes. Return the tube to the magnetic separator for 1 minute. Leaving the tube on the separator, transfer the eluate to a new microcentrifuge tube using a pipette.
   The eluate contains the purified PCR product.

7. Analyzing Results

7.A. **PCR Product Yield**

PCR product recovery can be determined by using a fluorometer with intercalating dye, gel electrophoresis analysis, and/or spectrophotometer.

7.B. **PCR Clean-Up Confirmation**

Gel electrophoresis can be used to evaluate the efficacy of the clean-up reaction. Using an agarose or PAGE gel, run a "before" sample of the pre-purified PCR product (Section 6.C.1) with an "after" sample of the cleaned-up product (Section 6.C.8), to ensure that < 100 bp primer dimers, reagents, etc. have been removed. **Figure 2** below shows an electrophoresis analysis of a PCR product using **AbraMag® PCR Clean-Up Magnetic Beads**. Note the total removal of material < 100 bp in the elution.

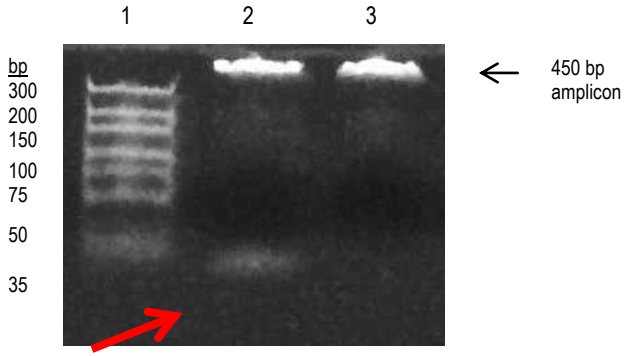

Figure 2. Gel electrophoresis analysis of 450 bp GAPDH PCR product purification using **AbraMag® PCR Clean-Up Magnetic Beads**. 10% PAGE gel, 100V for 20 minutes. Lane 1 – MW ladder; Lane 2 – PCR product with unwanted leftover reagents and dyes; Lane 3 – purified PCR product. Retesting of Lane 3 purified product showed high efficiency PCR re-amplification (data not shown), indicating that the purification process results in a high quality amplicon for downstream applications.