AbraMag® Clean-Up and Size Select 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® Clean-Up and Size Select Magnetic Beads

Product No. 544100 (5 mL)
544103 (60 mL)

1. General Description

AbraMag® Clean-Up and Size Select Magnetic Beads are designed to purify DNA fragments of a desired size out of a sheared DNA sample, amplicons for PCR clean-up, or similar samples for use in downstream applications such as cloning or sequencing. Fragments and amplicons are reversibly bound to paramagnetic particles while excess primers, salts, enzymes, and free nucleotides 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 2-8°C. **Do not freeze the magnetic beads**. Do not use after the printed expiration date.

4. Principle

The **AbraMag® Clean-Up and Size Select Magnetic Beads** process uses a simple, efficient, magnetic bead-based procedure for DNA fragment purification from a mixed reaction, as illustrated below in **Figure 1**:

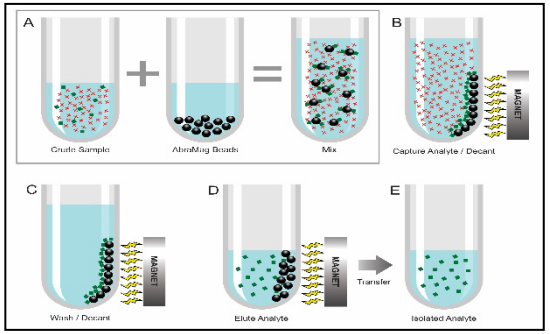

Figure 1. Schematic of the **AbraMag® Clean-Up and Size Select Magnetic Beads** process.

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

4.B. **Binding:** DNA fragments or amplicons of a desired size are captured by the beads in the presence of the optimized buffer. A magnet is used to secure the beads, with DNA attached.

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

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

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

5. Limitations and Precautions

Samples perform best when eluted or stored in molecular biology-grade water or Tris/TE solution prior to size selection or clean-up.

6. Working Instructions

6.A. **Materials Provided**

1. AbraMag® Clean-Up and Size Select Magnetic Beads

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

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

6.C. **Selecting Bead Ratio for Desired Size Selection**

The size cutoff, favoring retention of DNA fragments higher than 100 bp, can be controlled by altering the ratio of **AbraMag® Clean-Up and Size Select Magnetic Beads** to the starting sample volume. **Figure 2** below shows a gel electrophoresis analysis of size selection according to increasing ratios. Check **Table 1** for example ratios.

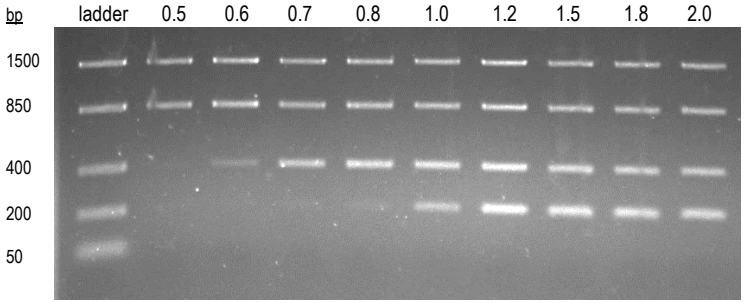

Figure 2. 1.5% agarose gel electrophoresis of DNA ladder showing effect of beads to sample ratios on fragment size exclusion. 100V for 25 minutes.

Ratio	Size Cutoff (bp)	Volume of beads (µL)
0.5	500 +	25
0.6	400 +	30
0.7	300 +	35
1.0	200 +	50
1.8	**100 +**	**90**

Table 1. Example bead ratios and resulting size selection using a starting sample of 50 µL. A ratio of 1.8 is recommended for PCR Clean-Up.

6.D. **Procedure**

1. Transfer the sample to be purified to a 1.5 mL microcentrifuge tube. Dilute the sample to at least 50 µL using molecular biology-grade water.
   Note: To use a larger volume of starting sample, simply multiply it by the desired ratio (Section 6.C).
   Ex: 100 µL sample x 1.8 = use 180 µL of beads in Section 6.D.2.
   Note: A small volume of starting product can be saved for gel analysis. See Section 7.B.
2. Vortex the **AbraMag® Clean-Up and Size Select Magnetic Beads** with at least 10 x 1 second pulses or rotate for at least 1 minute to ensure that the beads are completely re-suspended. *It is essential that bead solution is homogeneous!* Check Section 6.C to determine the volume of beads to use, according to the ratio for the desired size selection, and add that volume of beads to the sample. **For PCR Clean-Up, use a 1.8 ratio (90 µL beads to 50 µL sample).** Pipette up and down to mix well. It is recommended to try a range of ratios closest to the desired base pair size in order to optimize the fragment size selection.
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. Carefully remove all ethanol by pipette.
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 molecular biology-grade water to the sample. Pipette up and down to mix well. Incubate at room temperature for 2-3 minutes.
9. Return the tube to the magnetic separator for 1 minute. Leaving the tube on the separator, without disturbing the beads, transfer the eluate to a new microcentrifuge tube.
   The eluate contains the purified DNA fragments.

7. Analyzing Results

7.A. **Product Recovery**

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

7.B. **Clean-Up and Size Selection Confirmation**

Gel electrophoresis can be used to evaluate efficacy of the size selection, and to check optimization of the beads to sample ratio. Using an agarose or PAGE gel, run the eluted sample recovered in Section 6.D.9 compared to a molecular weight ladder to ensure that the desired fragment sizes have been selected.