## 7. Analyzing Results

### 7.A. **Plasmid DNA Yield**

Plasmid DNA yield can be estimated by UV absorbance. Using a spectrophotometer blanked against the DNA Elution Solution, measure the $A_{260}$ (DNA absorbance) reading. For DNA, an $A_{260}$ of 1.0 = 50 µg/mL when measured in a cuvette with a 10 mm optical path length. Use the following equation:

Plasmid DNA yield (µg) = $A_{260}$ reading x dilution factor x 50 µg/mL x sample elution volume (mL)

In some cases, subtracting the turbidity/cuvette impurity absorbance at $A_{320}$ from the $A_{260}$ reading may be necessary for a corrected reading that does not overestimate the DNA quantity.

Plasmid DNA yield can also be quantified using micro-fluorometers with specific intercalating dyes. This method is recommended when RNA may also be present in the eluted sample.

### 7.B. **Plasmid DNA Quality**

Similarly, plasmid DNA quality can be estimated by UV absorbance readings. Measure the absorbance at $A_{280}$ and $A_{230}$, and correct by subtracting the $A_{320}$ absorbance if necessary. Highly pure DNA has an $A_{260}/A_{280}$ ratio of ~1.7–2.0, indicating it has minimal contamination by proteins, and an $A_{260}/A_{230}$ ratio of >1.5, indicating it has minimal contamination by organic compounds and salts. Below, **Figure 2** displays a spectrophotometric curve and **Figure 3** gel electrophoresis of pUC19 plasmid DNA purified from ~1.0 x $10^9$ *E.coli* Top10 transformed cells.

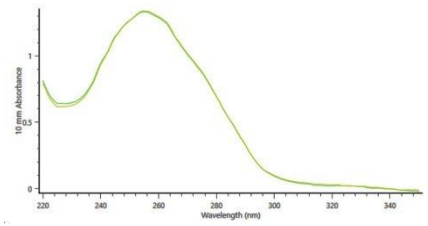


**Figure 2**. Spectrophotometric curve with an $A_{260}/A_{280}$ ratio of 1.8 and $A_{260}/A_{230}$ ratio of 2.0.

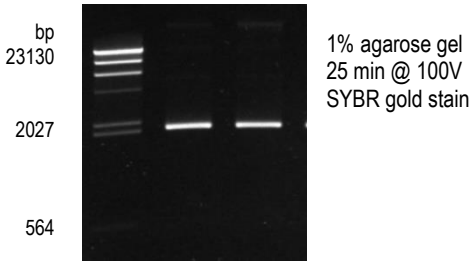


**Figure 3**. Lane 1–λHindIII MW ladder
Lane 2-3–purified pUC19 plasmid (2686 bp)
Multiple plasmid conformations (supercoiled, linear, etc.) may be visible.

**The AbraMag® Plasmid DNA Magnetic Purification Kit is intended for research and *in vitro* use only. This product was not tested or certified for diagnostic use.**

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For ordering or technical assistance contact:

Abraxis Inc.
124 Railroad Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
*Email:* info@abraxiskits.com
*WEB:* www.abraxiskits.com


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# AbraMag® Plasmid DNA Magnetic Purification Kit

**Product No. 555005 (100 purifications)**

## 1. General Description

The **AbraMag® Plasmid DNA Magnetic Purification Kit** is designed to purify plasmid DNA from high and low copy number *Escherichia coli* (*E.coli*) bacteria culture. Paramagnetic beads with uniform particle size efficiently bind plasmid DNA, resulting in high yields with minimal carryover of chromosomal DNA, proteins, nucleases, or other cellular contaminants. The kit is intended for manual purifications using a magnetic separator.

## 2. Safety Instructions

Use appropriate protective equipment (including but not limited to gloves, lab coats, and safety glasses) when culturing bacteria and using the kit. The Plasmid DNA Wash Solution 1 contains guanidine hydrochloride, which can be irritating to eyes and skin. DO NOT ADD ACIDS OR BLEACH to liquid waste or spills containing guanidine hydrochloride, as contact with acids or bleach can release toxic gases. **Refer to Safety Data Sheet for further information**.

## 3. Storage and Stability

Upon delivery of the kit, remove the RNase A Solution and store at -20°C. Once the RNase has been added to the Plasmid DNA Resuspension Solution, store the solution at 4°C (See Section 6.C.2 for details). Remove the **AbraMag® DNA Purification Magnetic Beads** and store at 4°C. **Do not freeze the magnetic beads solution**. All other kit reagents may be stored at room temperature (20-25°C). Do not use after the printed expiration date.

## 4. Kit Principle

The **AbraMag® Plasmid DNA Magnetic Purification Kit** process uses a simple, efficient, magnetic bead-based procedure for Plasmid DNA purification, as illustrated below in **Figure 1**:

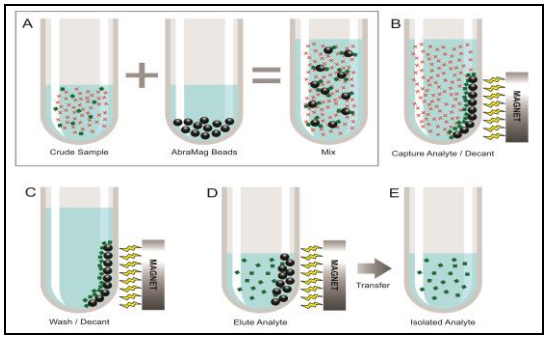


**Figure 1.** Schematic of **AbraMag® Plasmid DNA Magnetic Purification Kit** process.

4.A. **Sample Processing:** The *E.coli* sample is lysed to denature proteins and digest RNA, then neutralized to precipitate out chromosomal DNA. The neutralized sample is then added to the magnetic beads.

4.B. **Binding:** Plasmid DNA is captured by the beads in the presence of ethanol. A magnet is used to secure the beads, with DNA attached.

4.C. **Washing:** Remaining cell debris is washed away in a series of two wash steps.

4.D. **Elution:** DNA is then eluted and transferred to a new tube.

4.E. **Downstream Applications:** Pure, high-quality Plasmid DNA may then be used for downstream procedures such as PCR and enzymatic reactions; or stored long-term.

## 5. Limitations and Precautions

Overall DNA yield, quality and test reproducibility may vary depending on sample type and copy number, age, and condition before and after storage.

## 6. Working Instructions

6.A. **Materials Provided**

1. Plasmid DNA Resuspension Solution, 20 mL
2. RNase A Solution, 0.5 mL
3. Plasmid DNA Lysis Solution, 20 mL
4. Plasmid DNA Neutralization Solution, 20 mL
5. AbraMag® DNA Purification Magnetic Beads, 2 x 1 mL
6. Plasmid DNA Wash Solution 1 concentrate, 24 mL
7. Plasmid DNA Wash Solution 2 concentrate, 15 mL
8. DNA Elution Solution, 10 mL

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

1. Disposable gloves and other protective equipment
2. Micro-pipettes with disposable plastic aerosol barrier filter tips
3. 1.5 mL sterile plastic microcentrifuge tubes
4. 4°C refrigerator
5. -20°C freezer
6. 96-100% Ethanol
7. Luria-Bertani broth or other *E.coli* culture medium
8. Vortexer
9. Heating block, thermomixer, or water bath capable of 60°C
10. Magnetic microcentrifuge tube separator, Solo (Abraxis PN 472270) or Multi-6 (Abraxis PN 472260) or similar
11. Minicentrifuge

6.C. **Reagent Preparation**

1. Before the first use of the kit, add 96-100% Ethanol to the Plasmid DNA Wash Solution 1 concentrate and Plasmid DNA Wash Solution 2 concentrate as specified below. Mark the bottle to indicate that ethanol has been added. Wear gloves when handling the reagents (see Safety Instructions in Section 2).

    - Plasmid DNA Wash Solution 1: Add 36 mL 96-100% Ethanol
    - Plasmid DNA Wash Solution 2: Add 45 mL 96-100% Ethanol

2. Add the provided RNase A Solution to the Plasmid DNA Resuspension Solution and mix well. Store the solution at 4°C for up to 6 months. For longer storage, store RNase A separately at -20°C and only add to an aliquot of the Resuspension Solution immediately before kit use, at a ratio of 20 µL of RNase A per 1 mL of Resuspension Solution. Addition of RNase A can be omitted if removal of RNA is not required for downstream applications.

3. Before each use, check for any precipitate formation in the solutions. If observed, shake to re-dissolve any precipitates.

6.D. **Kit Procedure**

1. Grow transformed *E.coli* cells overnight (~16 hours, 150 rpm, 37°C) in Luria-Bertani culture medium with a compatible antibiotic. The optimal titer is ≥ 1.0 x $10^9$ cells.
2. Centrifuge > 1 mL of overnight culture at 5000 x g for 10 minutes in a microcentrifuge tube. Discard supernatant. Continue with the protocol or store the bacterial pellet at -20°C.
3. Resuspend the pelleted bacteria cells in 200 µL of **Plasmid DNA Resuspension Solution** (supplemented with RNase A, see section 6.C.2). Pipette up and down or vortex until homogenized.
4. Add 200 µL of **Plasmid DNA Lysis Solution** to the sample tube. Invert the tube gently ~5 times and incubate at room temperature for 2 minutes. *To prevent shearing of chromosomal DNA and denaturation of the supercoiled plasmid, do not vortex and do not let sample incubate longer than 2 minutes.*
5. Add 200 µL of **Plasmid DNA Neutralization Solution** to the sample tube**.** Gently invert the tube ~5 times to mix.
6. Add 100 µL of 96-100% Ethanol (not provided) to the sample tube. Gently invert the tube to mix.
7. Centrifuge the neutralized sample at 16,000 x g for 5 minutes to pellet chromosomal DNA and other cellular debris.
8. In a new 1.5 mL tube, add 300 µL of 96-100% Ethanol. Vortex the **AbraMag® DNA Purification Magnetic Beads** well to ensure complete resuspension of the beads, and add 20 µL of bead solution to the ethanol. Vortex well.
9. Transfer the ~600 µL centrifuged supernatant from Step 7 to the tube with the ethanol/bead mix in Step 8. Avoid pipetting any precipitates. Vortex for 3 seconds to mix well. Incubate at room temperature for 5 minutes.
10. Pulse spin ~1 second to remove any condensation/droplets from the sides and lid of the tube. Place the tube on the magnetic separator until supernatant has cleared, ~3 minutes. Leaving the tube on the separator, remove and discard the supernatant using a pipette, without disturbing the beads that have collected at the magnet.
11. Remove the tube from the magnetic separator and add 500 µL **Plasmid DNA Wash Solution 1** (prepared with ethanol, see section 6.C.1). Vortex well to resuspend the beads and return the tube to the magnetic separator for 2 minutes. Leaving the tube on the separator, remove and discard the supernatant using a pipette, without disturbing the beads that have collected at the magnet.
12. Remove the tube from the magnetic separator and add 500 µL **Plasmid DNA Wash Solution 2** (prepared with ethanol, see section 6.C.1). Vortex well to resuspend the beads and return the tube to the magnetic separator for 2 minutes. Leaving the tube on the separator, remove and discard the supernatant using a pipette, without disturbing the beads that have collected at the magnet.
13. Pulse spin to remove any wash solution drops remaining on the sides and lid of the tube. Return the tube to the magnetic separator for ~30 seconds. Leaving the tube on the separator, remove and discard any additional supernatant using a pipette, without disturbing the beads that have collected at the magnet. Make sure no wash solution remains in the tube.
    *Optional: Dry 5 minutes with cap open at room temperature if all wash solution has not been removed.*
14. Remove the tube from the magnetic separator and add 75 µL **DNA Elution Solution.** Vortex briefly to resuspend the beads.
15. Incubate the sample at 60°C for 5 minutes, vortexing occasionally or using a thermomixer.
16. If necessary, pulse spin to remove any condensation from the sides and lid of the tube.
17. Return the tube to the magnetic separator for 2 minutes. Leaving the tube on the separator, transfer the eluate to a new 1.5 mL tube using a pipette. ***The eluate contains the purified plasmid DNA.***

6.E. **Storing DNA**

Store the purified plasmid DNA in the DNA Elution Solution at 4°C for immediate use, or at -20°C for long-term storage. To avoid repeated freezing and thawing, store the DNA in aliquots.