

# SNARe™ Plasmid DNA Purification System

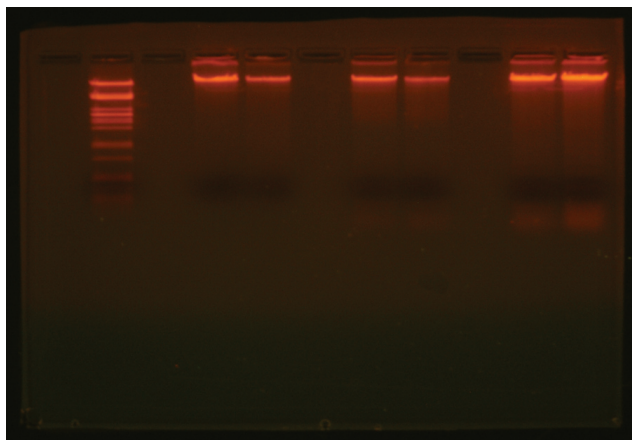
## Description

SNARe Plasmid DNA Purification System offers rapid, cost-effective methods for isolating DNA using DNA Separation Particles. DNA Separation Particles are a suspension of superparamagnetic iron oxide particles that bind double-stranded DNA. Once bound, the DNA-particle complex is stable and can be washed to remove any impurities or unwanted proteins from the sample to provide a clean DNA preparation. The DNA is eluted from the magnetic particles with an elution buffer for use in downstream reactions such as PCR, labeling, sequencing, transfection, cloning, and restriction digest.

**SNARe** (Simple Nucleic Acid Recovery) products are based on a new patent pending technology. SNARe offers the following advantages over other DNA purification methods:

- Easy-to-use procedure
- No need for columns or filters
- Reduces/eliminates repetitious centrifugation steps
- Reduces time required for DNA purification
- Eliminates use of toxic phenol/chloroform extraction of DNA
- Scalable - easily adjusts for sample size and automation
- DNA immediately available for PCR, restriction digestion

1 2 3 4 5 6 7 8 9 10 11



**Lane 1:** NA  
**Lane 2:** Molecular Weight Standards Lambda DNA PST-1  
**Lane 3:** NA  
**Lane 4:** Competitor Magnetic Separation Protocol  
**Lane 5:** Competitor Magnetic Separation Protocol  
**Lane 6:** NA; **Lane 7:** SNARe™ DNA Purification System  
**Lane 7:** SNARe™ DNA Purification System  
**Lane 8:** SNARe™ DNA Purification System  
**Lane 9:** NA  
**Lane 10:** SNARe™ DNA Purification System  
**Lane 11:** SNARe™ DNA Purification System

Figure 1

## Characteristics

Isolates:	Plasmids and cosmids
Sample Size:	1-3ml
Format:	Microcentrifuge tube
Number of Isolations per Kit:	100
Approximate Time Required:	<75 minutes for purification

## Material

### Material Supplied

SNARe Plasmid DNA Purification System contains DNA Separation Particles and all the buffers required to isolate DNA. Magnetic separators must be purchased separately, see "Magnetic Separators." All components are tested and are free of nucleases and contaminating DNA. *Note:* Store all solutions at 4°C, except where noted below.

- DNA Separation Particles (in Prep Buffer): 1.1ml  
*Note:* For handling instructions see the following section: Preparation of DNA Separation Particles.
- Prep Buffer: 3ml  
*Note:* Use to prewash DNA Separation Particles. Prewashing DNA Separation Particles is recommended for best results.
- Resuspension Buffer: 3.5ml  
*Note:* Prepares the bacteria in the sample for the Step: Lysing the Pellet.
- Plasmid Lysis Buffer: 7ml  
*Note:* Plasmid Lysis Buffer causes alkaline lysis of cells. Do not expose plasmids to the Plasmid Lysis Buffer longer than the recommended times to avoid extensive denaturation. Store at room temperature. Plasmid Lysis Buffer must be clear before use (all components dissolved) to allow cell lysis.
- Neutralization Solution: 5ml  
*Note:* Precipitates chromosomal DNA, denatured proteins, cellular debris, and SDS, which are subsequently removed from the sample.
- RNase A: 1.1ml  
*Note:* Removes RNA from the DNA preparation. Store at -20°C.
- DNA Binding Buffer: 1.5ml
- DNA Elution Buffer: 11ml  
*Note:* Elutes DNA from DNA Separation Particles.

### Material Required

- Centrifuge capable of 12,000 x G
  - 100% and 70% EtOH (in DEPC treated water)
- For microcentrifuge tube format:*
- Precision pipets with disposable tips to deliver 1-20µl, 20-200µl, 200-1000µl
  - 1.5ml or 2ml nuclease-free microcentrifuge tubes
  - One of the following magnetic separators:  
BioMag Solo-Sep Microcentrifuge Separator, single sample (Catalog Code 8MB4112S)  
BioMag Multi-6 Microcentrifuge Tube Separator, six samples (Catalog Code 8MB4111S)
- For the 96-well format:*
- Multi-well precision pipets with disposable tips to deliver 1-20µl, 20-200µl, 200-1000µl
  - Centrifuge for 96-well plates capable of 3500 rpm
  - 96-well rigid plate (1.2ml polypropylene wells)
  - BioMag 96-Well Plate Separator (Catalog Code 8MB4109S)
  - Plate sealer

## Procedure

Researchers are advised to optimize the use of particles in any application.

### A. Preparation of DNA Separation Particles

The DNA Separation Particles may be prewashed up to 24 hours in advance. Store at 4°C for up to 24 hours. After 24 hours, repeat the wash step prior to use. Prepare 10µl of DNA Separation Particles per sample prep. Scale according to the number and type of separations needed. (Recommendation for 96-well format: 10µl particles.)

For a single prep:

1. Shake the DNA Separation Particles bottle to ensure a uniform suspension.
2. Transfer the amount of DNA Separation Particles needed for the number of preparations desired to a microcentrifuge tube.
3. Add at least an equal volume of Prep Buffer.
4. Shake to ensure a uniform suspension.
5. Place the tube on a magnetic separator until the supernatant is clear.
6. While the tube is on the separator, aspirate and discard the supernatant.
7. Resuspend in Prep Buffer, to the original volume of bead suspension transferred.

#### *Helpful Tips for Aspirating Supernatant*

It is important not to contaminate the supernatant with particles. To aspirate the most supernatant without particles:

1. Hold the separator with the tube at eye level.
2. Tilt the tube such that the magnet wall of the separator faces up.
3. Hold the tube firmly against the wall. Angle the pipet tip away from the wall of the tube which the magnetic particles rest.

#### **B. Pelleting the Bacteria**

1. Add 1-3ml of culture to a microcentrifuge tube. *Note:* To process 3ml of sample, deliver 1.5ml of the sample into 2 microcentrifuge tubes. Combine pellets after centrifugation.
2. Centrifuge for 5 minutes at 5000 x G.
3. Carefully remove as much supernatant as possible and discard.
4. Air dry the pellet for 2 minutes.

#### **C. Lysing the Pellet**

1. Resuspend the pellet in 30 $\mu$ l of Resuspension Buffer.
2. Add 10 $\mu$ l of RNase A. Vortex the pellet into solution.
3. Add 60 $\mu$ l of room temperature Plasmid Lysis Buffer. Mix by shaking gently.
4. Incubate at room temperature for 5 minutes.
5. Add 45 $\mu$ l of Neutralization Solution. Mix by shaking gently.
6. Incubate on ice for 10 minutes.
7. Centrifuge for 5 minutes at 12,000-14,000 x G, at room temperature.
8. Carefully transfer 100 $\mu$ l of the supernatant containing the DNA to be isolated to a new microcentrifuge tube. Do not disturb the pellet at the bottom of the tube.

#### **D. Binding the DNA**

1. Add 10 $\mu$ l of washed DNA Separation Particles to the supernatant, mix by gentle inversion.
2. Add 11 $\mu$ l of DNA Binding Buffer to the supernatant and mix.
3. Add 300 $\mu$ l of 100% EtOH and mix by gentle inversion. Do not vortex.
4. Incubate at room temperature for 5 minutes with occasional mixing.
5. Place the tube on the magnetic separator until the supernatant is clear.
6. Leaving the tube on the separator, aspirate and discard the supernatant.
7. Wash the DNA Separation Particles by adding 500 $\mu$ l of 70% EtOH to the pellet and resuspend by gently pipetting to resuspend particles. Do not vortex.
8. Place the tube on the magnetic separator until the supernatant is clear.
9. Leaving the tube on the separator, aspirate and discard the supernatant.
10. Repeat Steps 7-9, two times.
11. Allow the pellet to air dry for approximately 5 minutes. Alternately, one may use a cotton swab to carefully absorb any liquid remaining on the side of the tube. Do not disturb the particles.

#### **E. Eluting the DNA**

*Note:* Do not substitute lab water for DNA Elution Buffer. Some lab water is less than pH 4.0. Low pH can interfere with downstream reactions involving enzymes.

1. Resuspend the DNA Separation Particles in 25-50µl of DNA Elution Buffer. Pipette mix. Do not vortex.
2. Incubate at room temperature for 5 minutes. Pipette mix.
3. Place the tube on a magnetic separator until the supernatant is clear.
4. While the tube is on the separator, transfer the DNA-containing supernatant to a clean tube.
5. Repeat Steps 1-4, combining the DNA supernatant fractions.

### DNA Yield

DNA obtained can be quantified on an agarose gel with expected yields of approximately 10µg/ml of culture.

### Storage and Stability

Store particles at 4°C. Freezing, drying, or centrifuging particles may result in irreversible aggregation and loss of binding activity.

### Safety

Before handling any chemicals in this System, refer to the Material Safety Data Sheets provided. Observe all relevant precautions, and follow all state, local, and federal regulations for chemical handling and disposal.

**This product is for research use only and is not intended for use in humans or for *in vitro* diagnostic use.**

### Ordering Information

Catalog Code	Description	Size
85081	SNARe™ Plasmid DNA Purification System	1 kit / 100 isolations

### Related Products

Catalog Code	Description	Size
85080	SNARe™ Genomic DNA Purification System (Technical Data Sheet 710)	1 kit / 100 isolations
85082	SNARe™ Plant Genomic DNA Purification System (Technical Data Sheet 712)	1 kit / 100 isolations

### Magnetic Separators

Catalog Code	Description	Size
8MB4112S	BioMag® Solo-Sep Microcentrifuge Tube Separator	1 each
8MB4111S	BioMag® Multi-6 Microcentrifuge Tube Separator	1 each
8MB4109S	BioMag® 96-Well Plate Separator	1 each

### To Order

In The U.S. Call: 1-800-523-2575 • 215-343-6484

In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767

In Germany FAX: (49) 6221-764620

Order online anytime at [www.polysciences.com](http://www.polysciences.com).