

SNARe™ Whole Blood Genomic DNA Purification System

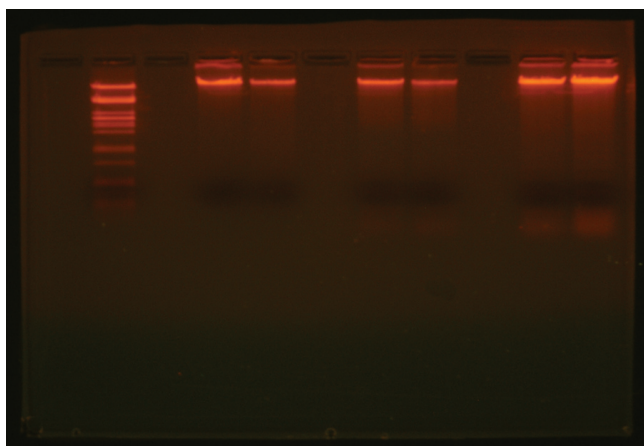
Description

SNARe Whole Blood Genomic 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 DNA-magnetic particle complex with an elution buffer and is ready 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
- Eliminates repetitious centrifugation steps
- Reduces the time required for DNA purification
- Eliminates the use of toxic phenol/chloroform extraction of DNA
- Scalable - easily adjusts for sample size and automation
- DNA is 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

Sample Source:	Human whole blood or white blood cells
Volume of Sample:	50-200µl (as little as 5µl for PCR applications)
Format:	Microcentrifuge tube
Number of Isolations per Kit:	100
Approximate Time Required:	50 minutes for purification

Material

Material Supplied

SNARe Whole Blood Genomic 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): 5.5ml
Note: For handling instructions, see the following section: Preparation of DNA Separation Particles.
- Prep Buffer: 11ml
Note: Use to prewash DNA Separation Particle. Prewashing DNA Separation Particles is recommended for best results.
- Whole Blood Lysis Buffer: 44ml
Note: After reconstitution, aliquot Whole Blood Lysis Buffer into microcentrifuge tubes and **store at -20°C** to avoid repeated freezing/thawing.
- DNA Binding Buffer: 7.2ml
- DNA Wash Solution: 106ml (352ml final volume)
Note: Washes away unwanted proteins to give a cleaner DNA preparation. Prior to use, add 246ml of 100% EtOH to the DNA Wash Solution. Mix well. Mark the label that ethanol has been added.
- DNA Elution Buffer: 22ml
Note: Elutes DNA from DNA Separation Particles.
- Proteinase K: 11mg
Note: Store at 4°C. When ready to use, reconstitute each with 0.5ml DEPC water to a 20mg/ml aliquot and **store at -20°C**.

Material Required

- 50°C Incubator
- Centrifuge capable of 12,000 x G (optional)
- 100% and 70% EtOH (in DEPC water)
- DEPC water

For microcentrifuge tube format:

- Precision pipets with disposable tips to deliver 1-20µl, 20-200µl, 200-1000µl
- 2ml nuclease-free microcentrifuge tubes
- One of the following magnetic separators:
BioMag Solo-Sep Microcentrifuge Tube Separator, single sample (Catalog Code 8MB4112S)
BioMag Multi-6 Microcentrifuge Tube Separator, six samples (Catalog Code 8MB4111S)

For the 96-well format (96-Well Microtiter Plate Protocol is available under Technical Data Sheet 710A):

- Multi-well precision pipets with disposable tips to deliver 1-20µl, 20-200µl, 20-1000µl
- Centrifuge for 96-well plates capable of 3500 rpm
- 2 x 96-well rigid plates
- 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 the quantity of DNA Separation Particles you will need according to the number and type of preps you will be performing. (Recommendation for 96-well format: 25µl particles.)

Suggested amounts of DNA Separation Particles

Whole blood or white blood cells, 2ml microcentrifuge tubes

Particles needed/prep

50µl

1. Shake the DNA Separation Particles bottle to ensure a uniform suspension.
2. Transfer the amount of DNA Separation Particles to a 2ml microcentrifuge tube. For example, if you are purifying DNA from 5 samples, transfer 250µl of DNA Separation Particles to a microcentrifuge tube.
3. Place the tube on a magnetic separator until the supernatant is clear.
4. While the tube is on the separator, aspirate and discard the supernatant.
5. Resuspend the particles in an equal volume of Prep Buffer. (If you started with 250µl of particles, add 250µl of Prep Buffer.)
6. Shake to ensure a uniform suspension.

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. Preparing Samples

Fresh Blood (Skip Steps 2-6) or White Blood Cells (water lysis or Ficoll hypaque is optional, use Steps 1-8)

1. Aliquot 50-200µl of blood into a 2ml microcentrifuge tube.
2. Add 1ml of nuclease-free deionized water.
3. Mix several times by inversion.
4. Centrifuge at 5000 x G for 2 minutes.
5. Discard the supernatant.
6. If needed, the pellet may be washed again with deionized water.
7. Resuspend in 400µl of Blood Lysis Buffer. Add 20µl Proteinase K and pipette mix.
8. Incubate at 50°C for 10 minutes.

If not prepared already, prewash DNA Separation Particles at this time.

C. Binding the DNA

If not already added, add 246ml of 100% EtOH to the DNA Wash Solution, as directed on the bottle label, and mix. Mark the label that ethanol has been added.

1. Add 50µl of washed DNA Separation Particles to the final supernatant from the section above, Preparing Samples.
2. Add 65µl of the DNA Binding Buffer and gently mix in 2ml microcentrifuge tubes.
3. Add 1.6ml of 100% EtOH to each tube and mix by gentle inversion.
4. Incubate at room temperature for 10 minutes with occasional gentle mixing.
5. Place the tubes on a magnetic separator until the supernatant is clear.
6. While the tubes are on the separator, aspirate and discard the supernatant.
7. First Wash Series:
 - a. Add 1.6ml of DNA Wash Solution to each sample tube. Resuspend the DNA-particle complex by pipette mixing. Do not vortex.
At this point the two sample tubes can be recombined into a single tube.
 - b. Place the tube on a magnetic separator until the supernatant is clear.
 - c. While the tube is on the separator, aspirate and discard the supernatant.
8. Repeat Step 7.
9. Second Wash Series (to be done with sample tube on or plate on the magnetic separator):
 - a. Add 1.6ml of 70% EtOH. Resuspend the DNA-particle complex by pipette mixing. Do not vortex.
 - b. Place the tube on a magnetic separator until the supernatant is clear.
 - c. While the tube is on the separator, aspirate and discard the supernatant.
10. Repeat Step 9, two times.
11. After removing residual ethanol, air dry the DNA Separation Particles for 2-5 minutes.

D. Eluting the DNA

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. Elute in 1-2x initial blood volume to rehydrate and elute bound DNA (200µl is recommended; no less than 100µl).
2. Resuspend the DNA Separation Particles in 200µl of DNA Elution Buffer by pipette mixing. Do not vortex.
3. Incubate at room temperature for 10 minutes. (Mixing optional.)
4. Place the tube on a magnetic separator until the supernatant is clear.
5. While the tube is on the separator, transfer the DNA contained supernatant to a clean tube.

E. DNA Yield

This procedure isolates 20µg of genomic DNA from 200µl of whole blood.

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
85080	SNARe™ Whole Blood Genomic DNA Purification System	1 kit / 100 isolations

Related Products

Catalog Code	Description	Size
85081	SNARe™ Plasmid DNA Purification System (Technical Data Sheet 711)	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

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