

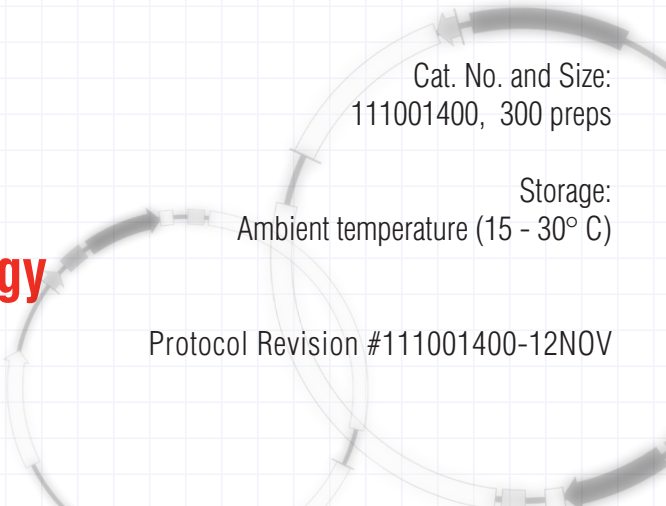


Application Manual

GeneClean[®] // Kit

*For rapid and user-friendly purification
of DNA in three basic steps*

- **One Call**
- **One Source**
- **A World of
Biotechnology
Reagents**



Cat. No. and Size:
111001400, 300 preps

Storage:
Ambient temperature (15 - 30° C)

Protocol Revision #111001400-12NOV

MSDS available online

mpbio.com/sampleprep



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1. Introduction

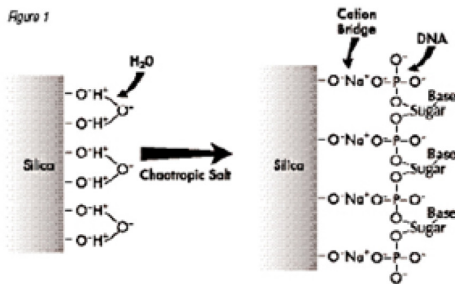
Patented GENECLEAN® technology simplifies the process of purifying DNA into three easy steps: Bind, Wash and Elute. Ethanol precipitation is never required and purified DNA is immediately ready for a wide variety of downstream applications. The GENECLEAN® II Kit is used to purify fragments of DNA from 200 bp to 20kb. The use of optional SPIN Filters allows isolation of fragments up to 300 kb.

1.1 Applications for GENECLEAN® Technology

- Desalting
- Isolate nucleic acids from agarose gels
- Eliminate proteins from enzymatic reactions
- Remove primers and unincorporated nucleotides from enzymatic reactions
- Separate linearized from uncut vector
- Isolate PCR product away from genomic DNA and primers

1.2 How Does GENECLEAN® Technology Work?

DNA generally binds to silica in high concentrations of chaotropic salt and elutes when the salt concentration is lowered. The mechanism of DNA binding to silica in high salt has not been completely described, but may involve chaotropic salt disruption of the water structure around negatively charged silica, allowing a cation bridge to form between it and the negatively charged phosphate backbone of DNA (fig. 1). When the salt concentration is lowered, rehydration of the silica matrix breaks the attraction between the matrix and DNA. The fact that DNA binds in high salt and elutes in low salt makes this method especially useful as a purification procedure. Since the DNA is eluted with either water or a low salt buffer, it can be used immediately in subsequent reactions without precipitation or other further manipulation. This is unlike ion exchange methods that require binding in low salt and elution in high salt and necessitate precipitation or other means of removing salt before the DNA can be used.



2. Kit Components and User Supplied Materials

2.1 GENECLEAN® II Kit Components

Nal (125 mL, Cat. #111001401) is supplied in a brown plastic bottle as a 6M solution.

GLASSMILK® (1.5 mL, Cat. #111001404) is a specially prepared aqueous suspension of proprietary silica matrix.

NEW Wash Concentrate (14 mL, Cat. #111001402) is a concentrated solution of NaCl, Tris, and EDTA to which water and ethanol are added to make NEW Wash (see Preparation of NEW Wash in Section 3.1). Store prepared NEW Wash at the bench (15°C-30°C), and keep tightly capped to prevent evaporation of ethanol.

TBE Modifier (15 mL, Cat. #111001403) is a proprietary mixture of concentrated salts for removing DNA from TBE-buffered gels: 0.5 volume of TBE Modifier and 4.5 volumes of Nal are added to 1 volume of agarose gel.

BioFloat™/Boil Away (2 Floats, Cat. #115021002) is a plastic float-rack for incubating 1.5 or 0.5 mL microcentrifuge tubes in a water bath. Tubes are held snugly with their tops above the surface of the water when the rack is floating.

2.2 Optional Components Available from MP Biomedicals, Inc.

SPIN Modules (100 Filters/Tubes, Cat. #112080800) can be purchased separately to provide maximum flexibility in DNA size range (200bp – 300kb) and are used to keep the GLASSMILK® on the surface of the filter while allowing liquid to flow through.

Label Block (1 mL, Cat. #111001605) is available to pre-treat the GLASSMILK® and help minimize irreversible binding of labeled DNA.

2.3 User Supplied Materials

1.5 mL microcentrifuge tubes

Clean bottle that accommodates up to 650 mL

100% ethanol

TE

Distilled water

Microcentrifuge

Heated water bath

Vortexer

3. Important Considerations Before Use

3.1 Preparation of NEW Wash

Pour contents of NEW Wash Concentrate into a bottle that accommodates at least 650 mL of liquid. Add 280 mL of distilled water and mix well. Add 310 mL of 100% (200 proof) ethanol and mix well. Do not use denatured alcohol because it can cause precipitation of salts. Label the prepared NEW Wash bottle with the label provided. Store tightly capped at 15°-30°C.

3.2 Possible Discoloration of Sodium Iodide (Nal)

When Nal is exposed to the air for a length of time, the solution (or a dried spill) will turn yellow. Nal contains a preservative to slow down this process, but if the bottle cap is left open the solution will discolor. The color change will not inhibit the function of Nal unless the pH, which normally ranges from 6.5 to 7.4, exceeds 7.4 High pH will cause a decrease in binding efficiency of DNA to GLASSMILK®. The pH can be lowered by adding 1/200 volume of 10% glacial acetic acid to the volume of Nal used or 1/30 volume of TBE Modifier. As the Nal solution is used, more air will be contained inside of the bottle after each use, even when the cap is closed tightly. Transferring the Nal or aliquoting it into smaller amber containers after receiving it will minimize exposure to air.

3.3 Reconstitution of GLASSMILK®

Particles of silica matrix on the cap of the container can prevent an airtight seal that will result in the evaporation of the liquid in the GLASSMILK® vial. If this occurs, reconstitute the GLASSMILK® by adding sterile, distilled water to the container so that the amount of liquid and solid is approximately equal. Mix well to fully activate GLASSMILK®.

3.4 Yield Measurements

The best method for checking yields of DNA isolated by GENE CLEAN® is to run an aliquot on an agarose gel using known quantities in adjacent lanes as controls. OD260 and fluorescent readings can also be used to estimate yields, but these methods are affected by trace amounts of salts and silica matrix, so it is best to confirm these readings by gel analysis.

3.5 Agarose Types

Low-melt agarose is not required for any GENE CLEAN®-based kit. The procedure will work with any molecular biology-grade agarose.

3.6 TBE Buffers

The use of the provided TBE Modifier alleviates the inhibitory effect TBE and other borate-containing buffers can have on the binding of DNA to silica.

3.7 Tips for Working with Smaller DNA

When working with DNA from 200-500 bp, it is recommended that 1/200 volume of 10% glacial acetic acid or 1/30 volume of TBE Modifier be added to the NaI. This will lower the pH of the NaI/GLASSMILK®/DNA mixture to approximately 6.0-6.5, where smaller DNA species will bind with maximum efficiency. When working with DNA greater than 500 bases in length, it is not necessary to make this adjustment. There is reported evidence that increasing the temperature during binding to 55°C will increase the binding efficiency of smaller DNA fragments.

3.8 Purifying Radio-Labeled DNA

Radioactive isotope-labeled DNA may bind irreversibly to GLASSMILK®. Label Block solution is available from MP Biomedicals (Cat #111001605) to pre-treat the GLASSMILK® and help minimize irreversible binding of labeled DNA. 1 µL of Label Block should be added for every 10 µL GLASSMILK®.

4. Simplified Protocols for Experienced Users

4.1 Purifying DNA from Solutions

1. Measure DNA volume.
2. Add 3 volumes NaI solution to DNA.
3. Calculate amount of GLASSMILK® needed.
4. Resuspend GLASSMILK® by vortexing for 1 minute.
5. Add GLASSMILK® to the DNA/NaI solution and mix.
6. Incubate at room temperature for 5 minutes and mix.
7. Pellet the GLASSMILK® with the bound DNA and discard supernatant.
8. Add 500 µl prepared NEW Wash and resuspend.
9. Centrifuge for 5 seconds and discard supernatant.
10. Repeat wash (Steps 8-9).

11. Dry pellet to remove residual ethanol.
12. Add a volume of TE or water equal to the amount of GLASSMILK® added in Step 5. Resuspend pellet to mix and elute DNA.
13. Centrifuge for 30 seconds and remove supernatant containing DNA.

4.2 Purifying DNA from TAE or TBE Agarose Gels

1. Excise DNA band from agarose gel.
2. Determine the weight of the gel slice in micrograms.
3. Determine approximate volume of gel slice (100 mg = 100 μ L).
4. Transfer gel slice to microcentrifuge tube.
5. For TAE gels, add 3 volumes Nal solution. For TBE gels, add 0.5 volume TBE Modifier and 4.5 volumes Nal solution per 1 volume of agarose.
6. Incubate gel slice and Nal solution at 55°C for 1 minute to melt gel.
7. Mix contents of tube.
8. Continue incubation until agarose is dissolved (5 minutes).
9. Calculate amount of GLASSMILK® needed.
10. Resuspend GLASSMILK® by vortexing for 1 minute.
11. Add GLASSMILK® to Nal/DNA solution as calculated in Step 9 and mix.
12. Incubate at room temperature for 5 minutes and mix.
13. Pellet the GLASSMILK® with the bound DNA and discard supernatant.
14. Add 500 μ l prepared NEW Wash and resuspend.
15. Centrifuge for 5 seconds and discard supernatant.
16. Repeat wash (Steps 14-15).
17. Dry pellet to remove residual ethanol.
18. Add a volume of TE or water equal to the amount of GLASSMILK® added in Step 11. Resuspend pellet to mix and elute DNA.
19. Centrifuge for 30 seconds and remove supernatant containing DNA.

5. Detailed Protocols

5.1 Purifying DNA from Solutions

1. Measure DNA volume. Approximate the volume of DNA in microliters or measure with a pipet.
2. Add 3 volumes of Nal solution. In a polypropylene centrifuge tube, add a volume of Nal to the DNA that is three times the volume of the starting DNA solution. This keeps the final concentration of Nal above 4M.
[Note: Do not use glass tubes or pipets to which DNA will bind.]

- Calculate amount of GLASSMILK® needed.
Determine amount of GLASSMILK® suspension required according to the considerations below:

Final Volume	Max. Amount of DNA	GLASSMILK®
<500 µL	<5 µg	5 µL
500-1000 µL	<7.5 µg	10 µL
1 mL	<12.5 µg	20 µL
3 mL	<50 µg†	100 µL

The amount of GLASSMILK® required is based on the amount of DNA and the volume of NaI solution. 1 µL of GLASSMILK® will bind 1-2 µg of DNA. Since a DNA molecule must collide with a silica particle to bind, keeping the volume low and the particles suspended will maximize binding efficiency.

† For larger amounts of DNA, see GENE CLEAN® Large Protocol in section 9.3.

- Resuspend GLASSMILK® by vortexing for 1 minute. When working with radio-labeled DNA, add 1 µL of Label Block (Cat. #111001605) to each 10 µL of GLASSMILK® and incubate for 5 minutes at room temperature before adding to the DNA/NaI solution in the next step.
- Add GLASSMILK® to the DNA/NaI solution as calculated in Step 3. Vortex gently or stir with a pipet tip to mix.
- Incubate at room temperature for 5 minutes. This allows binding of the DNA to the silica matrix. Mix every 1-2 minutes to ensure that GLASSMILK® stays in suspension.

[Note: If the volume of binding matrix is greater than 1 ml, incubate for at least 15 minutes while frequently mixing by hand, mechanical rocker, rotation wheel, or vortex at medium speed to keep the silica particles in suspension.]

- Pellet the GLASSMILK® with the bound DNA. Centrifuge at 14,000 x g for 5 seconds. Discard the supernatant. If the suspension is in a larger tube, spin it in a benchtop or other centrifuge at 14,000 xg for 1-2 minutes to pellet. SPIN Option: After incubation as detailed in Step 6, carefully transfer suspension to a SPIN Filter and centrifuge at 14,000 x g for 1 minute or until liquid is transferred to the Catch Tube. If working with volumes >750 µL, empty the Catch Tube and repeat centrifugation as needed.

8. Add 500 μ L prepared NEW Wash and resuspend.* Carefully resuspend the pellet by pipetting up and down while stirring the pellet with the pipet tip. The consistency of the pellet is different in prepared NEW Wash than in aqueous solutions and is somewhat resistant to resuspension. If the suspension is in a larger tube, resuspend the pellet in 500 μ L of NEW Wash and transfer to 1.5 mL microcentrifuge tube.

*VERY IMPORTANT: Be sure ethanol and water have been added to the NEW Wash Concentrate before using. See Section 3.1 for instructions.

SPIN Option: Add the prepared NEW Wash to the GLASSMILK® on the SPIN Filter and mix gently.

9. Centrifuge at 14,000 x g for 5 seconds and discard supernatant.

SPIN Option: Centrifuge at 14,000 x g until the wash solution is in the Catch Tube (1-3 minutes).

10. Repeat Wash Repeat the wash procedure once (as detailed in Steps 8-9).

SPIN Option: Repeat the wash procedure once (as detailed in Steps 8-9).

11. Dry the pellet to remove residual ethanol. After the supernatant from the final wash has been removed, centrifuge the tube again at 14,000 x g for a few seconds and remove the last bit of liquid with a small bore pipet tip to avoid diluting the eluate with prepared NEW Wash. Residual ethanol can interfere with many downstream reactions (i.e. ligations, sequencing) and elution efficiency and should be removed from the GLASSMILK® as thoroughly as possible.

[Note: The GLASSMILK®/DNA complex can be further dried to eliminate the small amount of ethanol that might be trapped in the void volume. Leave the cap open for 5-10 minutes at room temperature or 55°C in a heat block or water bath, or place the tube under a vacuum for 2-5 minutes.]

SPIN Option: Empty Catch Tube and centrifuge at 14,000 x g for 1 minute to dry the GLASSMILK®/DNA complex.

12. Add a volume of water or TE equal to the amount of GLASSMILK® calculated in Step 3. Carefully resuspend the pellet by gently pipetting up and down with wide-bore pipet tip or by tapping the side of the tube with a finger to mix and elute DNA.

SPIN Option: Transfer SPIN filter to a new Catch Tube and add water or TE equal to the amount of GLASSMILK® calculated in Step 3. Carefully resuspend the pellet by gently pipetting up and down with wide-bore pipet tip or by tapping the side of the tube with a finger.

13. Centrifuge at 14,000 x g for 30 seconds to pellet. Carefully remove supernatant containing the eluted DNA and place in a new tube. Approximately 80% of the bound DNA will elute in this first step. Save the tube containing the pellet until recovery of DNA has been verified.

[Note: A second elution is not necessary or recommended. Repetition of this step will cause the total volume to increase and the concentration of DNA to decrease.]

SPIN Option: Centrifuge at 14,000 x g for 1 minute or until all the liquid containing the DNA is in the Catch Tube. Discard the SPIN Filter.

5.2 Purifying DNA from TAE or TBE Agarose Gels

1. Excise the DNA band from the agarose gel. Excise the DNA band from an ethidium bromide-stained agarose gel with a razor blade using long wave UV light for as short a time as practical. Be sure to wear UV protection such as a face shield or goggles.
2. Determine the weight of the gel slice in micrograms.
3. Determine the approximate volume of the gel slice. 100 mg = 100 μ L
4. Transfer gel slice to a polypropylene tube. Do not use glass tubes or pipets to which the DNA will bind. If the gel slice weighs less than 400 μ g, use a 1.5 mL microcentrifuge tube. If the gel slice weighs more than 400 μ g, use a larger tube.

[Note: It is not necessary to crush the gel but large slices can be cut into 2 mm cubes to facilitate dissolving the gel during the next step.]

5. Add 3 volumes of Nal solution. Add a volume of Nal to the DNA that is three times the volume of the gel slice. This keeps the final concentration of Nal above 4M.

[Note: When using TBE gels, add 0.5 volume of TBE Modifier and 4.5 volumes of Nal per 1 volume of agarose.]

6. Incubate gel slice and NaI solution to melt agarose. Place the tube in a 45°-55°C water bath for 1 minute. The plastic BioFloat™ contained in the GENE CLEAN® II Kit can be used for this purpose.
7. Mix the contents of the tube by tapping the side with a finger or gently pipetting with a wide-bore pipet tip.
8. Continue the incubation until all of the agarose has dissolved (approximately 5 minutes).
9. Calculate amount of GLASSMILK® needed. Determine amount of GLASSMILK® suspension required according to the considerations below:

Final Volume	Max. Amount of DNA	GLASSMILK®
<500 µL	<5 µg	5 µL
500-1000 µL	<7.5 µg	10 µL
1 mL	< 12.5 µg	20 µL
3 mL	<50 µg†	100 µL

The amount of GLASSMILK® required is based on the amount of DNA and the volume of NaI solution. 1 µL of GLASSMILK® will bind 1-2 µg of DNA. Since a DNA molecule must collide with a silica particle to bind, keeping the volume low and the particles suspended will maximize binding efficiency. † For larger amounts of DNA, see GENE-CLEAN® Large Protocol in section 9.3.

10. Resuspend GLASSMILK® by vortexing for 1 minute. When working with radio-labeled DNA, add 1 µL of Label Block (Cat. #111001605) to each 10 µL of GLASSMILK® and incubate for 5 minutes at room temperature before adding to the DNA/NaI solution in the next step.
11. Add GLASSMILK® to NaI/DNA solution as calculated in Step 9 and mix. Vortex gently or stir with a pipet tip to mix.
12. Incubate at room temperature for 5 minutes. This allows binding of the DNA to the silica matrix. Mix every 1-2 minutes to ensure that GLASS MILK® stays in suspension.

[Note: If the volume of binding matrix is greater than 1 ml, incubate for at least 15 minutes while frequently mixing by hand, mechanical rocker, rotation wheel, or vortex at medium speed to keep the silica particles in suspension.]

- 13 Pellet the GLASSMILK® with the bound DNA. Centrifuge at 14,000 x g for 5 seconds. Discard the supernatant. If the suspension is in a larger tube, spin it in a benchtop or other centrifuge at 14,000 xg for 1-2 minutes to pellet.

[Note: When using >1.5% gels or when the agarose does not dissolve completely, the pellet should be resuspended with 200-400 µL of NaI stock solution. Place suspension in the 45°-55°C water bath for a few minutes and then pellet by spinning in a microcentrifuge for 5 seconds at full speed. Discard NaI wash supernatant. This step is usually not necessary if care is exercised in initially dissolving all of the agarose.]

SPIN Option: After incubation as detailed in Step 12, carefully transfer suspension to a SPIN Filter and centrifuge for 1 minute (<14,000 x g) or until liquid is transferred. If working with volumes >750 µL, empty the catch tube and repeat wash centrifugation as needed.

14. Add 500 µL prepared NEW Wash and resuspend.* Carefully resuspend the pellet by pipetting up and down while stirring the pellet with the pipet tip. The consistency of the pellet is different in prepared NEW Wash than in aqueous solutions and is somewhat resistant to resuspension. If the suspension is in a larger tube, resuspend the pellet in 500 µL of NEW Wash and transfer to 1.5 mL microcentrifuge tube.

*VERY IMPORTANT: Be sure ethanol and water have been added to the NEW Wash Concentrate before using. See Section 3.1 for instructions.

SPIN Option: Add the prepared NEW Wash to the GLASSMILK® on the SPIN Filter and mix gently.

15. Centrifuge at 14,000 x g for 5 seconds and discard supernatant.

SPIN Option: Centrifuge at 14,000 x g until the wash solution is in the Catch Tube (1-3 minutes).

16. Repeat Wash Repeat the wash procedure once (as detailed in Steps 14-15).

SPIN Option: Repeat the wash procedure once (as detailed in Steps 14-15).

17. Dry the pellet to remove residual ethanol. After the supernatant from the final wash has been removed, centrifuge the tube again at 14,000 x g for

a few seconds and remove the last bit of liquid with a small bore pipet tip to avoid diluting the eluate with prepared NEW Wash. Residual ethanol can interfere with many downstream reactions (i.e. ligations, sequencing) and elution efficiency and should be removed from the GLASSMILK® as thoroughly as possible.

[Note: The GLASSMILK®/DNA complex can be further dried to eliminate the small amount of ethanol that might be trapped in the void volume. Leave the cap open for 5-10 minutes at room temperature or 55°C in a heat block or water bath, or place the tube under a vacuum for 2-5 minutes.]

SPIN Option: Empty Catch Tube and centrifuge at 14,000 x g for 1 minute to dry the GLASSMILK®/DNA complex.

18. Add a volume of water or TE equal to the amount of GLASSMILK® calculated in Step 9. Carefully resuspend the pellet by gently pipetting up and down with wide-bore pipet tip or by tapping the side of the tube with a finger to mix and elute DNA.

SPIN Option: Transfer SPIN filter to a new Catch Tube and add water or TE equal to the amount of GLASSMILK® calculated in Step 9. Carefully resuspend the pellet by gently pipetting up and down with wide-bore pipet tip or by tapping the side of the tube with a finger.

19. Centrifuge at 14,000 x g for 30 seconds to pellet. Carefully remove supernatant containing the eluted DNA and place in a new tube. Approximately 80% of the bound DNA will elute in this first step. Save the tube containing the pellet until recovery of DNA has been verified.

[Note: A second elution is not necessary or recommended. Repetition of this step will cause the total volume to increase and the concentration of DNA to decrease.]

SPIN Option: Centrifuge at 14,000 x g for 1 minute or until all the liquid containing the DNA is in the Catch Tube. Discard the SPIN Filter.

6. Common Questions

6.1 Do I need to do anything different when using GENE CLEAN® II with high molecular weight DNA (>10 kb)?

Shearing can be a problem for high-molecular-weight DNA, but may be minimized by doing the following:

1. Use a wide-bore pipet and minimize agitations of the GLASSMILK®/DNA pellet. For the wash steps, allow the GLASSMILK® pellet to soak in the NEW Wash instead of resuspending it. When eluting, gently resuspend the pellet using a wide-bore pipet.
2. Use SPIN Modules (Cat. #2080-800) with the GENE CLEAN® II Kit to avoid shearing the DNA while it is bound to the GLASSMILK®.

6.2 Does GENE CLEAN® II work on all conformations of plasmid DNA?

Yes. If yields are not satisfactory, increasing the binding time and the volume of GLASSMILK® will generally increase recoveries. However, our line of Plasmid Purification Kits would be best-suited for purification of plasmid DNA. See Section 10 for related products.

6.3 Can I substitute GENE CLEAN® II NEW Wash Concentrate with other GENE CLEAN® Wash Solutions?

No. The wash solutions have different salt concentrations and are prepared differently.

6.4 If I'm using GENE CLEAN® II, what do I do if I have more than 50 µg of DNA or a large gel slice?

If working with more than 5 µg of DNA, add 2 µL of GLASSMILK® for every 1 µg of DNA and proceed with the written protocol. A large sample can be divided and processed in parallel according to the GENE CLEAN® protocols. An alternate protocol for processing large gel slices (>1 g) or large amounts of DNA (>50 µg) can be found in Section 9.3.

7. Troubleshooting

7.1 Low or No Recovery with the GENE CLEAN® II Kit

7.1.1 Problems with Binding

Binding of the DNA is dependent on both salt concentration and pH. If less than 3 volumes of NaI were used, the salt concentration might not be high enough to allow the DNA to bind to the GLASSMILK®. As NaI gets older or is used frequently, it can change color and the pH may change. Once the pH rises above 7.5, the binding efficiency decreases. Adjust pH with glacial acetic acid or TBE Modifier as described in Section 3.2. Yields can also be affected by insufficient incubation and mixing time during the GLASSMILK®/DNA binding step. Consistent gentle mixing at this step can increase the efficiency by as much as 50%.

Radioactive isotope-labeled DNA may bind irreversibly to the silica. Label Block is available separately for use with any GENE CLEAN® Kit. See Section 3.8 for details.

The GENE CLEAN® Kits are designed specifically for purification of DNA of length >200 bp. Qbiogene offers the MERmaid® Kit for purification of DNA of length <200 bp. See Section 10.1 for ordering information.

7.1.2 Problems with Washing

DNA may elute in the wash if ethanol was not added to the NEW Wash Concentrate prior to use. Prepare NEW Wash as described Section 3.1. If the NEW Wash ethanol concentration drops significantly due to evaporation, the DNA may elute in the wash. Store the prepared NEW Wash tightly capped at 15-30°C.

7.1.3 Problems with Elution

DNA will elute from the GLASSMILK® in water, TE, or other low-salt, neutral solutions. If residual NEW Wash is present, the yield may be low and the residual ethanol may interfere with many downstream applications. Be sure to remove all traces of NEW Wash by drying the pellet for 5 minutes prior to elution. A second elution can be done resulting in an additional 10-20% recovery of eluted DNA but this step is not necessary or recommended.

7.1.4 Rapid Kit Reagent – Test Procedure

If yields are less than 50%, this test takes 15-20 minutes to determine if the problem is due to reagents or to some other aspect of the procedure.

1. Put 0.5-1 µg of DNA into a final volume of 20 µL H₂O or TE buffer. Transfer 10 µL into a microcentrifuge tube.
2. Add 30 µL NaI solution to this tube and mix.
3. Transfer 10 µL (1/4 of the total volume) to a second tube. To precipitate the DNA: add 30 µL of water and 60 µL of isopropanol and spin for 5 minutes. Drain the tube and add 10 µL of water.
4. Add 5 µL GLASSMILK® to the remaining 30 µL of DNA/NaI.
5. Incubate at room temperature with mixing to keep the GLASSMILK® suspended.
6. After 5 minutes, pellet the GLASSMILK®/DNA by centrifugation.
7. Transfer the NaI supernatant to another tube and precipitate as in Step 3.

8. Resuspend the pellet in 300 μL prepared NEW Wash. Centrifuge again to pellet the GLASSMILK[®].
9. Save the NEW Wash.
10. Resuspend the pellet in 10 μL TE or H₂O. Pellet and save supernatant.
11. Repeat elution (Step 10) a second time. Save this supernatant in a new tube.
12. Run the samples on a 0.8% agarose minigel as shown below until they migrate 1-2 cm.

Lane 1: 7.5 μL DNA from the 10 μL left in step 1 that was not purified with GENE CLEAN[®]. This equals the amount of DNA that was purified.

Lane 2: 10 μL first elution (step 10)

Lane 3: 10 μL second elution (step 11)

Lane 4: 10 μL NEW Wash (step 9, add Ficoll[®], sucrose, or glycerol to keep the NEW Wash in the well).

Lane 5: GLASSMILK[®] pellet from step 11 resuspended in running buffer.

Lane 6: 10 μL of the precipitated NaI supernatant after GLASSMILK[®] absorption (step 7)

Lane 7: 10 μL of the precipitated NaI/DNA solution before GLASSMILK[®] is added (This is the 10 μL aliquot removed in step 3 before adding GLASSMILK[®] and contains 1/3 the quantity of DNA that was exposed to GLASSMILK[®]). The results of the gel should show the fate of DNA during the GENE CLEAN[®] procedure. Most of the DNA should be in the first elution (lane 2), some (approximately 10%) should be in the second elution (lane 3), and none in NEW Wash or NaI after absorption (lanes 4 and 6, respectively). If DNA is seen in lane 6, this indicates that not all of the DNA bound to the GLASSMILK[®]. DNA in lane 4 indicates loss during the NEW Wash step. The relative quantities of DNA in each elution will indicate efficiencies during this step. If there is any DNA that would not elute from the GLASSMILK[®] by diffusion, it may do so by electroelution (lane 5). (Note: GLASSMILK[®] will fluoresce slightly in the well. This is not DNA). The results of this rapid kit test normally show a recovery of 70% or more in the first elution.

7.1.5 Problems Measuring Yield

DNA yield can be quantified with a fluorometer or estimated by running the sample against a known amount of DNA on an agarose gel. Using a spectrophotometer to quantify DNA yield is not recommended for the following reasons:

1. Residual silica particles (which do not interfere with downstream reactions or uses of the DNA) can scatter UV light, affecting OD260 readings and OD260/OD280 ratios.
2. After diluting part of your sample up to the minimum volume of the cuvette, the DNA will often be too dilute to give a significant reading. For example, if you eluted 0.5 µg in 20 µL of water and diluted 2 µL of this to 200 µL, the final concentration of DNA in the cuvette would be $0.5 \mu\text{g}/0.02 \text{ ml} \times 0.01 = 0.25 \mu\text{g}/\text{mL}$. This would give an absorbance of only 0.005, which is too low to be significant on most instruments.

7.2 Possible Discoloration of Sodium Iodide (Nal)

When Nal is exposed to the air for a length of time, the solution (or a dried spill) will turn yellow. Nal contains a preservative to slow down this process, but if the bottle cap is left open the solution will discolor. The color change will not inhibit the function of Nal unless the pH, which normally ranges from 6.5 to 7.4, exceeds 7.4. High pH will cause a decrease in binding efficiency of DNA to GLASSMILK®. The pH can be lowered by adding 1/200 volume of 10% glacial acetic acid to the volume of Nal used or 1/30 volume of TBE Modifier. As the Nal solution is used, more air will be contained inside of the bottle after each use, even when the cap is closed tightly. Transferring the Nal or aliquoting it into smaller amber containers after receiving it will minimize exposure to air.

7.3 Reconstitution of GLASSMILK®

Particles of silica matrix on the cap of the container can prevent an airtight seal that will result in the evaporation of the liquid in the GLASSMILK® vial. If this occurs, reconstitute the GLASSMILK® by adding sterile, distilled water to the container so that the amount of liquid and solid is approximately equal. Mix well to fully activate GLASSMILK®.

7.4 GLASSMILK® Particles in Eluted DNA

It is often difficult to remove the last bit of eluate from the top of the pellet without carrying over a small amount of the insoluble silica matrix. DNA will not bind to silica in less than 3M salt, so the carried-over matrix will not normally interfere with subsequent use of the DNA. However, it has been suggested that silica particles may sometimes interfere with

PCR. As a precaution to avoid transferring residual silica, centrifuge the tube at 14,000 x g of eluted DNA for a few seconds before removal of an aliquot from the upper part of the liquid.

7.5 Replacing NEW Wash Solution

The amount of NEW Wash provided is sufficient for 300 preps when the protocol is followed as recommended. If the protocol is changed such that more than 2 mL of prepared NEW Wash Solution are required per prep, a solution of 50% ethanol and 100 mM NaCl, prepared using TE (pH 7.5), will work nearly as well as the proprietary NEW Wash Solution.

7.6 Binding of Lower Molecular Weight Fragments

When working with DNA from 200-500 bp, it is recommended that 1/200 volume of 10% glacial acetic acid or 1/30 volume of TBE Modifier be added to the NaI. This will lower the pH of the NaI/GLASSMILK®/DNA mixture to approximately 6.0-6.5, where smaller DNA species will bind with maximum efficiency. When working with DNA greater than 500 bases in length, it is not necessary to make this adjustment. There is reported evidence that increasing the temperature during binding to 55°C will increase the binding efficiency of smaller DNA fragments.

8. Recommended Reference Format

DNA was purified from gel or solution using the GENECLEAN® II Kit (MP Biomedicals, Inc., Carlsbad, California).

9. Supplemental Protocols

9.1 Rapid Isolation of Phage ssDNA

The GENECLEAN® procedure can be incorporated into small-scale, single-stranded bacteriophage DNA isolation protocols. Because phenol/chloroform is usually used to lyse phage, the GENECLEAN® process helps to rid the final DNA preparation of these solvents. The cleaned solution is less inhibitory when added to polymerase or other enzyme reaction mixtures, thus helping to optimize sequencing results.

1. Pellet cells from 1.5 mL of a cell culture producing M13 or other ssDNA phage.
2. Transfer 1 mL of supernatant to new tube. Avoid transferring any host cells to prevent contamination with host cell DNA and RNA.
3. Add PEG solution (20% PEG 8000, 2.5 M NaCl, pH 3.5). Incubate at

room temperature for 10 minutes and spin for 10 minutes to precipitate phage.

4. Resuspend small phage pellet in 50 μ L of TE.
5. Lyse phage in one of two ways:
 - a. Add an equal volume of buffer-saturated phenol, vortex briefly, and spin for 2 minutes. Remove upper phase.
 - b. Add 50 μ L of formamide, mix, and heat at 55°C for 15 minutes.
6. Proceed with GENE CLEAN® II Protocol for purifying DNA from solution (Section 5.1) to purify the ssDNA.

9.2 Eliminate BAP, CIP, SAP

After dephosphorylation reactions, incubate reaction tube at 75°C for 15 minutes in a water bath and follow the GENE CLEAN® II protocol for purifying DNA from solution (Section 5.1) to eliminate dephosphorylation enzymes.

9.3 GENE CLEAN® Large (>50 μ g DNA)

1. Add 3 volumes of Nal to the DNA and incubate at room temperature for 30 minutes.
 - a. Determine the volume. For gels, excise band from agarose gel and determine the weight of the gel slice. Place into a 50 mL conical centrifuge tube.
 - b. Add 3 volumes of Nal (3 mL/g gel slice).
 - c. For gels, place on rotating wheel or shaker for 30 minutes or until gel is completely dissolved.
2. Add GLASSMILK®; allow DNA to bind from several hours to overnight, spin for 2 minutes.
 - a. Add 4 μ L of GLASSMILK® for every μ g of DNA.
 - b. Bind DNA by placing tube on rotating wheel for several hours or overnight. It is important that the GLASSMILK® remain in suspension for efficient binding to take place.
 - c. Spin the tube in a table-top centrifuge at 3,000 x g for 2 minutes and decant the supernatant.
3. Wash with 5 mL of NEW Wash Solution, spin for 2 minutes.
 - a. Add 5 mL of room temperature NEW Wash and let soak for 5 minutes. Do not disturb pellet.
 - b. Spin for 2-4 minutes in a table-top centrifuge at 3,000 x g and decant the supernatant.
 - c. Pulse spin and use a pipet to remove a residual NEW Wash that may be present.
 - d. Air dry for 5 minutes.

4. Elute DNA in 2 volumes of water.
 - a. Resuspend the GLASSMILK® pellet in 2 volumes of water.
 - b. Place in 55°C water bath for 5 minutes.
 - c. Spin for 2-4 minutes in a table-top centrifuge at 3,000 x g and carefully transfer the supernatant containing your DNA to a new 2.0 mL microcentrifuge tube.
5. Isopropanol precipitate, wash with 80% ethanol, resuspend in desired volume of water.
 - a. Add NaCl to a final concentration of 0.2M and 0.7 volumes of Isopropanol to the eluted DNA.
 - b. Mix well and spin in a microfuge at full speed for 5 minutes. Decant supernatant. (Some residual agarose will precipitate at this point. It will not interfere with your DNA recovery.)
 - c. Add 500 µL of 80% ethanol to pellet and place on rotating wheel or shaker for 5-10 minutes. Pulse centrifuge and decant supernatant.
 - d. Pulse spin and remove residual ethanol. Let air dry 5 minutes.
 - e. Resuspend pellet in desired volume of water. Place at 55°C for 5 minutes. Spin for 2 minutes and transfer supernatant containing the DNA to a new tube.

10. Related Products

10.1 Gel Isolation and Reaction Cleanup Products

Cat. No.	Description	Size
111102200	GENECLEAN® Turbo Kit	50 preps
111102400	GENECLEAN® Turbo Kit	100 preps
111103200	GENECLEAN® Turbo for PCR Kit	50 preps
111103400	GENECLEAN® Turbo for PCR Kit	100 preps
111001200	GENECLEAN® Kit	200 preps
111001400	GENECLEAN® II Kit	300 preps
111001600	GENECLEAN® III Kit	600 preps
111101200	GENECLEAN® SPIN Kit	50 preps
111101400	GENECLEAN® SPIN Kit	100 preps
111104200	GENECLEAN® Turbo 96 Kit	96 preps
111104400	GENECLEAN® Turbo 96 Kit	384 preps
111005200	MERmaid® Kit	200 preps
111105200	MERmaid® SPIN Kit	25 preps
111105400	MERmaid® SPIN Kit	75 preps
111105600	MERmaid® SPIN Kit	150 preps
111007200	RNaid® Kit	200 preps

111107200	RNaid® SPIN Kit	200 preps
119903100	SeqDirect™ PCR Cleaning Kit	16 reactions
119903200	SeqDirect™ PCR Cleaning Kit	32 reactions
119904200	SeqDirect™ 96 PCR Cleaning Kit	1 x 96-well plate
112350200	EtBr GREENBAG™ Disposal Kit	50 bags
112080600	SPIN Module (Includes #2080-601)	60 F/T
112080800	SPIN Module (Includes #2080-801)	100 F/T
111001605	Label Block	1 mL

10.2 GENE CLEAN®-Based Genomic DNA Isolation Kits

Cat. No.	Description	Size
116540400	FastDNA® Kit	100 preps
116560200	FastDNA® Kit for Soil	50 preps
112010400	GNOME® DNA Isolation Kit	25 preps
112010600	GNOME® DNA Isolation Kit	100 preps
112011600	GNOME® Whole Blood DNA Isolation Kit	100 preps
112012400	FLORACLEAN® Kit	25 preps
111002200	GENE CLEAN® for Ancient DNA Kit	100 preps
112016200	Whole Cell Yeast PCR Kit	200 preps
112015600	Yeast Cell Lysis Kit	100 preps
112055400	λQuick!® Kit	25 preps
112065200	ssPHAGE™ DNA SPIN Kit	60 preps

10.3 Plasmid Purification Products

Cat. No.	Description	Size
112066200	RapidPURE™ Plasmid Mini Kit	60 preps
112066400	RapidPURE™ Plasmid Mini Kit	120 preps
112066600	RapidPURE™ Plasmid Mini Kit	300 preps
112067200	RapidPURE™ Plasmid Mini 96 Kit	96 preps
112067400	RapidPURE™ Plasmid Mini 96 Kit	192 preps
112070200	RPM® Kit	60 preps
112070400	RPM® Kit	120 preps
112070500	RPM® Kit	300 preps
112069400	Yeast RPM® Kit	100 preps
112000200	MiniPrep Express™ Matrix	1,250 preps
112002200	96well Prep Express	384 preps

11. Product Use Limitation & Warranty

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Application Manual

GENECLEAN® // Kit

Protocol Revision # 111001400-12NOV

Cat. No. 111001400, 300 preps

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