GENECLEAN® Kit

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

Revision # 1001-999-3E05

GENECLEAN® Kit

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

Application Manual

Revision # 1001-999-3E05

Cat. No. 1001-200
200 preps

Storage:
Ambient temperature (15–30°C)

TABLE OF CONTENTS

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® Kit is used to purify fragments of DNA from 200 bp to 20 kb. The use of optional SPIN Filters allows isolation of fragments up to 300 kb.

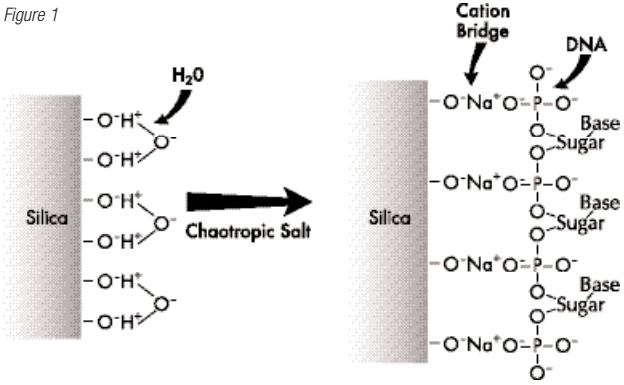
1.1 Applications for GENECLEAN® Technology

- 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.

Figure 1



2. Kit Components and User Supplied Materials

2.1 GENECLEAN® Kit Components

NaI (60 ml, Cat. #1001-201) is supplied in a brown plastic bottle as a 6M solution.

GLASSMILK® (1.25 ml, Cat. #1001-204) is a specially prepared aqueous suspension of proprietary silica matrix that has been treated so that it can be easily resuspended by hand.

NEW Wash Concentrate (7 ml, Cat. #1001-202) 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.

BioFloat™/Boil Away (2 Floats, Cat. #5021-002) 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 Qbiogene, Inc.

SPIN Modules (100 Filters/Tubes, Cat. #2080-800) 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. #1001-605) is available to pre-treat the GLASSMILK® and help minimize irreversible binding of labeled DNA.

TBE Modifier (15 ml, Cat. #1001-403) 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 NaI are added to 1 volume of agarose gel.

2.3 User Supplied Materials

1.5 ml microcentrifuge tubes
Clean bottle that accommodates up to 350 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 350 ml of liquid. Add 140 ml of distilled water and mix well. Add 155 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 (NaI)

When NaI is exposed to the air for a length of time, the solution (or a dried spill) will turn yellow. NaI 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 NaI 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 NaI used or 1/30 volume of TBE Modifier.

As the NaI solution is used, more air will be contained inside of the bottle after each use, even when the cap is closed tightly. Transferring the NaI 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 GENECLEAN® is to run an aliquot on an agarose gel using known quantities in adjacent lanes as controls. OD_{260} 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 GENECLEAN®-based kit. The procedure will work with any molecular biology-grade agarose.

3.6 TBE Buffers

The use of TBE Modifier alleviates the inhibitory effect TBE and other borate-containing buffers can have on the binding of DNA to silica. See Section 2.2 for details.

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 Qbiogene (Cat #1001-605) 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 µl).
4. Transfer gel slice to microcentrifuge tube.
5. For TAE gels, add 3 volumes NaI solution. For TBE gels, add 0.5 volume TBE Modifier and 4.5 volumes NaI solution per 1 volume of agarose.
6. Incubate gel slice and NaI solution at 55°C 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 NaI/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 µl 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 NaI solution.
   In a polypropylene centrifuge tube, add a volume of NaI to the DNA that is three times the volume of the starting DNA solution. This keeps the final concentration of NaI above 4M.
   [***Note:*** Do not use glass tubes or pipets to which DNA will bind.]

3. 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 GENECLEAN® Large Protocol in section 9.3.

4. Resuspend GLASSMILK® by vortexing for 1 minute.
   When working with radio-labeled DNA, add 1 µl of Label Block 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.

5. Add GLASSMILK® to the DNA/NaI solution as calculated in Step 3.
   Vortex gently or stir with a pipet tip to mix.

6. 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.]

7. 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 x g 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. If working with volumes >750 µl, empty the Catch Tube and repeat centrifugation as needed.

8. 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.

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 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 µl

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 µg, use a 1.5 ml microcentrifuge tube. If the gel slice weighs more than 400 µg, 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 NaI solution.
   Add a volume of NaI to the DNA that is three times the volume of the gel slice. This keeps the final concentration of NaI above 4M.
   [***Note:*** When using TBE gels, add 0.5 volume of TBE Modifier and 4.5 volumes of NaI per 1 volume of agarose. TBE Modifier is available separately. See Section 2.2 for details.]

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 GENECLEAN® 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 GENECLEAN® 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 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 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.]

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 x g 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 200-400µl 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 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 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 SPIN Filter.

6. Common Questions

6.1 Do I need to do anything different when using the GENECLEAN® Kit 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 GENECLEAN® Kit to avoid shearing the DNA while it is bound to the GLASSMILK®.

6.2 Does GENECLEAN® 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 GENECLEAN® NEW Wash Concentrate with other GENECLEAN® Wash Solutions?

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

6.4 If I'm using GENECLEAN®, 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 GENECLEAN® protocols. Alternatively, a special GENECLEAN® 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 GENECLEAN® 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 GENECLEAN® Kit. See Section 3.8 for details.

The GENECLEAN® 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 there is residual NEW Wash 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 H_2O 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 H_2O. 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 GENECLEAN®. 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 GENECLEAN® 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 GLASSMIK®. 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 OD_{260} readings and OD_{260}/OD_{280} 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 µg/0.02 ml x 0.01=0.25 µg/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 (NaI)

When NaI is exposed to the air for a length of time, the solution (or a dried spill) will turn yellow. NaI 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 NaI 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 NaI used or 1/30 volume of TBE Modifier.

As the NaI solution is used, more air will be contained inside of the bottle after each use, even when the cap is closed tightly. Transferring the NaI 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

Occasionally, the amount of NEW Wash provided is insufficient for each researcher's individual needs. Although the composition of NEW Wash Concentrate is proprietary, a solution of 50% ethanol using TE (pH 7.5) and 100 mM NaCl will work nearly as well.

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® Kit (Qbiogene, 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 µl 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 µl of formamide, mix, and heat at 55°C for 15 minutes.
6. Proceed with GENECLEAN® Protocol for purifiying 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 GENECLEAN® protocol for purifying DNA from solution (Section 5.1) to eliminate dephosphorylation enzymes.

9.3 GENECLEAN® Large (>50µg DNA)

1. Add 3 volumes of NaI to your 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 NaI (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 µl of GLASSMILK® for every µg 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 an 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
1102-200	GENECLEAN® Turbo Kit	50 preps
1102-400	GENECLEAN® Turbo Kit	100 preps
1103-200	GENECLEAN® Turbo for PCR Kit	50 preps
1103-400	GENECLEAN® Turbo for PCR Kit	100 preps
1001-200	GENECLEAN® Kit	200 preps
1001-400	GENECLEAN® II Kit	300 preps
1001-600	GENECLEAN® III Kit	600 preps
1101-200	GENECLEAN® SPIN Kit	50 preps
1101-400	GENECLEAN® SPIN Kit	100 preps
1104-200	GENECLEAN® Turbo 96 Kit	96 preps
1104-400	GENECLEAN® Turbo 96 Kit	384 preps
1005-200	MERmaid® Kit	200 preps
1105-200	MERmaid® SPIN Kit	25 preps
1105-400	MERmaid® SPIN Kit	75 preps
1105-600	MERmaid® SPIN Kit	150 preps
1007-200	RNaid® Kit	200 preps
1107-200	RNaid® SPIN Kit	200 preps
9903-100	SeqDirect™ PCR Cleaning Kit	16 reactions
9903-200	SeqDirect™ PCR Cleaning Kit	32 reactions
9904-200	SeqDirect™ 96 PCR Cleaning Kit	1 x 96-well plate
2350-200	EtBr GREENBAG™ Disposal Kit	50 bags
2300-604	50xTAE “GENECLEAN Grade™” Electrophoresis Buffer	1.0 L
2305-204	TBE “GENECLEAN Grade™” Electrophoresis Buffer Mix	425 g
2305-304	TBE “GENECLEAN Grade™” Electrophoresis Buffer Mix	1,700 g
2080-600	SPIN Module (Includes #2080-601)	60 F/T
2080-800	SPIN Module (Includes #2080-801)	100 F/T
1001-605	Label Block	1 mL

10.2 GENECLEAN®-Based Genomic DNA Isolation Kits

Cat. No.	Description	Size
6540-400	FastDNA® Kit	100 preps
6560-200	FastDNA® Kit for Soil	50 preps
2010-400	GNOME® DNA Isolation Kit	25 preps
2010-600	GNOME® DNA Isolation Kit	100 preps
2011-600	GNOME® Whole Blood DNA Isolation Kit	100 preps

2012-400	FLORACLEAN® Kit	25 preps
1002-200	GENECLEAN® for Ancient DNA Kit	100 preps
2016-200	Whole Cell Yeast PCR Kit	200 preps
2015-600	Yeast Cell Lysis Kit	100 preps
2055-400	λQuick!® Kit	25 preps
2065-200	ssPHAGE™ DNA SPIN Kit	60 preps

10.3 Plasmid Purification Products

Cat. No.	Description	Size
2066-200	RapidPURE™ Plasmid Mini Kit	60 preps
2066-400	RapidPURE™ Plasmid Mini Kit	120 preps
2066-600	RapidPURE™ Plasmid Mini Kit	300 preps
2067-200	RapidPURE™ Plasmid Mini 96 Kit	96 preps
2067-400	RapidPURE™ Plasmid Mini 96 Kit	192 preps
2070-200	RPM® Kit	60 preps
2070-400	RPM® Kit	120 preps
2070-500	RPM® Kit	300 preps
2069-400	Yeast RPM® Kit	100 preps
2000-200	MiniPrep Express™ Matrix	1,250 preps
2002-200	96well Prep Express	384 preps
2005-200	RapidPURE™ Plasmid Midi Kit	25 preps
2005-400	RapidPURE™ Plasmid Midi Kit	75 preps
2005-600	RapidPURE™ Plasmid Midi Kit	150 preps
2076-200	RapidPURE™ Plasmid Maxi GF Kit	20 preps
2073-200	RapidPURE™ Plasmid Maxi GF Reagent Kit	20 preps
2074-200	RapidPURE™ Plasmid Maxi GF Endo Free Kit	10 preps
2078-200	RapidPURE™ Plasmid Giga Kit	12 preps

10.4 TRIPLE CHECK® Tips

Cat. No.	Description	Size
5030-121	TRIPLE CHECK® Tips (non-sterile)	Bag of 500
5030-141	TRIPLE CHECK® Tips (non-sterile)	Bag of 1,000
5030-151	TRIPLE CHECK® Tips (non-sterile)	Bag of 5,000
5030-221	TRIPLE CHECK® Tips (non-sterile)	Box of 250
5030-241	TRIPLE CHECK® Tips (non-sterile)	Box of 500
5030-321	TRIPLE CHECK® Tips (non-sterile)	4 racks of 96
5030-331	TRIPLE CHECK® Tips (non-sterile)	10 racks of 96
5030-322	TRIPLE CHECK® Tips (sterile)	4 racks of 96
5030-332	TRIPLE CHECK® Tips (sterile)	10 racks of 96

11. Product Use Limitation & Warranty

Unless otherwise indicated, this product is for research use only. Purchase of Qbiogene, Inc. products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. Qbiogene, Inc. makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and the sole liability of Qbiogene, Inc. hereunder shall be limited to, at our discretion, no replacement or compensation, product credits, refund of the purchase price of, or the replacement of materials that do not meet our specification. By acceptance of the product, Buyer indemnifies and holds Qbiogene, Inc. harmless against, and assumes all liability for, the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refund or replacement is conditioned on Buyer notifying Qbiogene, Inc. within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

NOTES

NOTES