BIO 101 Protocol

web

MERmaid® Kit

With Optional **SPIN™** Procedure Protocol Included

Catalog Number	Prep Size
1005-000	10 preps
1005-200	200 preps
Optional	
2080-400	40 Filters/Catch Tubes
2080-600	60 Filters/Catch Tubes
2080-800	100 Filters/Catch Tubes

- **Complete System for Oligomer and PCR Product Purification**
- **Size Range 10-200 Nucleotides.**
- **Works Equally Well with ssDNA and dsDNA.**
- **Purify DNA from Solution or Gels - Agarose or Polyacrylamide.**

Shipping & Storage:

The **MERmaid®** kit is shipped and stored at ambient temperature.

"Check with BIO 101... We have you time in mind!"

BIO 101

Revision #: *1005-999-8F05P*

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MERmaid® Protocol

Kit Components

1005-000 (Sample Size, not for sale)

Name	Volume	Cat #
High Salt Binding Solution	6 ml	1005-001
GLASSFOG®	200 µl	1005-004
Ethanol Wash Concentrate*	1.5 ml	1005-002
50x Electrophoresis Buffer**	4 ml	1005-005
Biogel	2.25 g	1005-003

***Add 13.5 ml of 100% ethanol to the* Ethanol Wash Solution and mix before initial use.**

****Dilute the stock 50 fold with water before use.***

1005-200

Name	Volume	Cat #
High Salt Binding Solution	60 ml	1005-201
GLASSFOG®	1.5 ml	1005-204
Ethanol Wash Concentrate*	15 ml	1005-202
50x Electrophoresis Buffer**	100 ml	1005-205
Biogel	5 g	1005-203

****The Ethanol Wash Solution is prepared by adding the contents of the 15 ml bottle to 135 ml of 100% ethanol.***

****Dilute the stock 50 fold with water before use.***

Optional Components

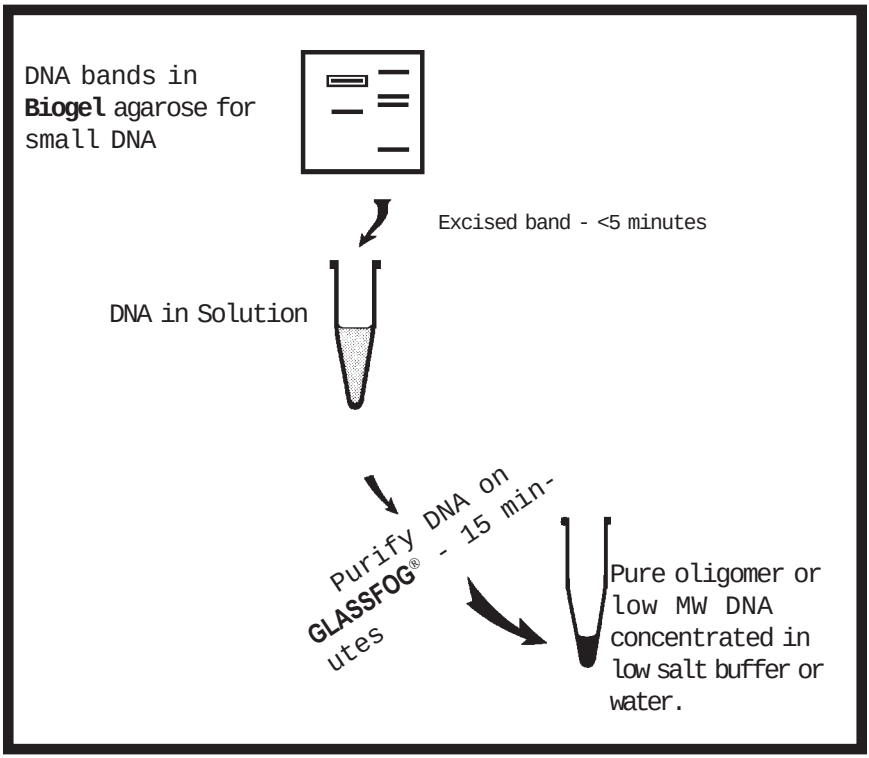
Name	Quantity	Cat #
SPIN™ Module	40 Filters	2080-400
SPIN™ Module	60 Filters	2080-600
SPIN™ Module	100 Filters	2080-800

All references to user supplied reagents are italicized and bolded in the protocol for your convenience.

Introduction

The **MERmaid**® Kit is designed as a **Complete System** to rapidly purify small quantities of low molecular weight single and double stranded DNA oligomers. The Kit will remove and purify DNA between 10 bp and 200 bp from agarose or solution. When purifying DNA from agarose, especially if <50bp in length, we highly recommend that all **MERmaid**® kit reagents be used. Substituting other agaroses or electrophoresis buffers may result in less than optimum yields. In any case, do not use TBE buffers. (For more efficient recovery of DNA species greater than 200 bp in length use one of the **GENECLEAN**® Kits from **BIO 101**).

The MERmaid〉 Process



Protocol

To Purify Oligomers from Solution

1. • Determine volume of DNA solution.
   • Add **3 volumes High Salt Binding Solution**.
2. • Vortex **GLASSFOG®** vial until resuspended. Add **5-8 µl GLASSFOG®** per µg of DNA. Mix.
   • Incubate at room temperature for 5-15 minutes.
   • Efficient binding is dependent upon vigorous mixing during this incubation period. Significant increases in yield, especially with shorter length species of DNA, are often obtained by vortexing the tube during the entire binding period.
3. • Centrifuge in a microcentrifuge at high speed for a few seconds to pellet the **GLASSFOG®**.
   • Remove supernatant and save aside.

Option: Wash pellet with **200 µl High Salt Binding Solution**. *Spin tube for one or two seconds and remove last bit of with supernatant small bore pipet. When purifying DNA from agarose, this wash is specially useful for removing traces of agarose.*

4. • Add **300 µl Ethanol Wash (**be sure to reconstitute as per instruction on label prior to initial use) and resuspend the **GLASSFOG®** pellet by vortexing for a few seconds. The wash effectively removes salts or other compounds that will inhibit enzymes.
   • Centrifuge briefly and discard supernatant.
   • Repeat **Ethanol Wash** step (#4) one or two more times. After last wash, spin tube for a second or two and remove remaining liquid with small bore pipet.

Option: Pellet can be dried under vacuum for a few minutes to remove traces of ethanol.

5. • Elute oligomer DNA from **GLASSFOG®** by resuspending pellet in a small volume of water. A convenient elution volume is equal to the volume of **GLASSFOG®** added in step 2.
   • Incubate at 45-55° C for 5 minutes.
   • Centrifuge for 1 minute.
   • Transfer supernatant to a new tube.
   • Repeat elution step once and combine the two elutions.

Running low MW DNA in Agarose

The **MERmaid**® Kit contains a sample of **Biogel** agarose and a special gel electrophoresis buffer to ensure excellent resolution of low MW DNA bands and enable optimal recoveries of DNA. **Biogel** is a **BIO 101** product consisting of a highly pure agarose formulation designed to be less brittle than other types of agarose capable of resolving low MW DNA bands.

To resolve DNA species less than 50 bp in length use 4-6% **Biogel** agarose. For DNA 50 to 100 bp use 3% **Biogel**; for longer than 100 bp use 2 % **Biogel.**

To Conserve Biogel Agarose

Cast a 1% agarose gel using any high quality DNA electrophoresis grade agarose. Cut out and remove a section of the gel below one lane, including well, for each sample you will be testing. Cast a **Biogel** (equal in volume to the section removed) into the resulting frame after removing the cut section. Ethidium bromide (0.5 μg/ml) can be added to the agarose before casting. Gels wrapped in Saran Wrap can be stored at 4° C for several days before or between uses.

When casting and running these agarose gels, use the **Electrophoresis Buffer** supplied in the kit for optimal recovery of DNA from the gel, especially if 50bp or less in length. Dilute the 50x concentrated solution to 1x with good quality water before use. Enough 50x concentrate is included in the kit to make 5 liters (200 ml with sample size kit) of 1x **Electrophoresis Buffer**. If other electrophoresis buffers are used, soak the gel slice in a solution of 1 x **Electrophoresis Buffer** for 15 minutes prior to extracting the DNA with the **MERmaid**® kit. Avoid TBE buffers, if possible, because efficient recovery of DNA from agarose is adversely affected by the presence of borate. Gels run in the presence of 1/2 to 1 μg/ml ethidium bromide will allow visualization of single stranded DNA.

Removal of low MW or oligomer DNA from Agarose

1. Use **Biogel** and **Electrophoresis Buffer** for best results. For sharper resolution bands, limit electrophoresis to 10-15 minutes at high voltage to minimize diffusion of DNA in agarose. Cut the desired DNA band from agarose gel and place in a microcentrifuge tube. Add **3 volumes High Salt Binding Solution**.

2. Add **8 µl** resuspended **GLASSFOG**® per µg of DNA in tube. (Binding capacity is approximately 1 µg DNA per µl of **GLASSFOG**® , but it is best to add excess to increase binding kinetics.)
   Vortex tube continuously for 10 minutes. The gel will "melt" rapidly at room temperature and the DNA will bind to the **GLASSFOG**® most efficiently under vigorous mixing conditions. Continue...with step 3 (page 5). (In the protocol for removing oligomers from solution, be sure to include the optional **High Salt Binding Solution**.)

To remove oligomer DNA from Polyacrylamide Gels:

• Method A: Cut out the band and cut or crush the gel into small pieces. Soak the gel pieces in approximately twice the volume of **High Salt Binding Solution** for 20 minutes at 60° C. Centrifuge and remove the supernatant avoiding gel pieces. Repeat soak with one volume **High Salt Solution** and pool supernatants. Add **GLASSFOG**® and continue with the **MERmaid**® kit protocol for solutions. Continue with step 3 (page 5)

• Method B: Cut out the band from the polyacrylamide gel and place into a slit cut in a **Biogel** agarose gel. Electroelute the DNA from the polyacrylamide into the **Biogel** agarose. Excise the band from the **Biogel** and purify the DNA using the **MERmaid**® procedure. (Step 1, page 6).

MERmaid® with SPIN™ Module Protocol

In a microcentrifuge tube:

1) Add **3 volumes High Salt Binding Solution** to DNA.

   a) If working with solution, add **3µl High Salt Binding Solution** per µl of DNA solution.
   b) If working with agarose, add **3**µl **High Salt Binding Solution** per mg of gel slice.

2) Add **GLASSFOG**®; vortex; add suspension to a **SPIN™ Filter**; Centrifuge.

   a) Vortex **GLASSFOG**® until homogenous.
   b) Add **5-8µl GLASSFOG**® suspension per µg of DNA.
   c) Vortex tube continuously for 10 minutes to melt gel and allow for DNA binding. (Do not transfer suspension to filter until after vortexing because silica particles will tear **SPIN™ Filter**).
   d) Transfer suspension to a **SPIN™ Filter**. The **SPIN™ Filter** capacity is 750µl, multiple spins are necessary when working with larger volumes of suspension.
   e) Spin in a microcentrifuge at 14,000 xg for 15 seconds, longer if necessary, to transfer liquid to **Catch Tube**. (For this and all wash steps, empty **Catch Tube** as needed.)

Note: Wash pellet with **200µl High Salt Binding Solution** *when removing DNA from agarose.*

3) Wash twice with **Ethanol Wash**.

   a) Add **300µl Ethanol Wash** (be sure to follow reconstitution instructions on the **Ethanol Wash** Label) solution to the **SPIN™ Filter**.
   b) Spin for 30 seconds at 14,000 xg or until **SPIN™ Filter** is emptied of **Wash.** Repeat wash.
   c) Empty **Catch Tube** and spin for 1 minute to “dry” pellet of residual ethanol that might interfere with subsequent reactions.

4) Transfer **SPIN™ Filter** to a clean **Catch Tube** and elute DNA with ***H_2O*** or ***TE***.

a) Add an amount that is 1-2 times the volume of the **GLASSFOG**〉 suspension used in 2a. and gently resuspend the white pellet by hand, pipet, or vortex. (If vortexing - do not exceed 1-2 seconds at 1/2 speed).

b) Spin tube for 30 seconds at 14,000 xg to transfer eluted DNA to **Catch Tube**.

A second elution can increase yield by 10-20%.

c) Discard **SPIN™ Filter** and cap the tube. DNA in solution is ready to use without further manipulation.

Troubleshooting Hints

Recovery efficiencies are usually lower out of agarose than solution. If recoveries are unsatisfactory or less than 50%:

1. Check supernatant from binding step for DNA by adding more **GLASSFOG**® and repeating the **MERmaid**® procedure. Pay particular attention to mixing during the binding step.

2 If you did not use the **Biogel** agarose and **Electrophoresis Buffer** reagents supplied with the kit, try repeating the procedure using these components.

Notes

Notes

Product Use Limitation & Warranty

Unless otherwise indicated, this product is for research use only. Purchase of **BIO 101** products does not grant rights to reproduce, modify, repackage the products or any derivative thereof to third parties. **BIO 101** 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 **BIO 101**'s sole liability hereunder shall be limited to, at our option, product credits, refund of the purchase price of, or the replacement of all material(s) that does not meet our specification. By acceptance of the product, Buyer indemnifies and holds **BIO 101** 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 refunds or replacement is conditioned on Buyer notifying **BIO 101** within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (days) shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

General Information

BIO 101 is a pioneer in developing kits for molecular biology research. We introduced the **GENECLEAN**® Kits in 1986 and have since been manufacturing products to bring convenience into your research. Our goal is to make your life easier by simplifying the complexities of lab work.

Technical Support and Ordering Information

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