BIO 101 Protocol

GENECLEAN® Kit For Ancient DNA

Catalog Number	Prep Size
1002-100	10 preps
1002-200	100 preps

- DNA Isolation from Museum or Ancient Specimens for PCR
- Isolate DNA from Samples of Bone, Preserved Tissue or Animal By-products
- Resulting Samples are Readily Amplified by PCR or other Methods
- Each Prep is Capable of Isolating 20 µg DNA

Shipping & Storage:

The **GENECLEAN® Kit for Ancient DNA** is shipped and stored at ambient temperature.

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

BIO 101

Revision #: *1002-999-6J03P*

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GENECLEAN® for Ancient DNA Protocol

Kit Components

1002-100 (10 preps)

Name	Volume	Catalog #
Dehybernation Solution A*	11 ml	1002-101
Dehybernation Solution B	11 ml	1002-109
Dehyb Solution A_2	1.8 ml	1002-111
Ancient DNA **GLASSMILK**®*	4 ml	1002-102
Salton Wash #1*	6 ml	1002-103
Salton Wash #2	6 ml	1002-108
Ancient DNA Alcohol Wash**	1.5 ml	1002-104
DNA-free Elution Solution	1.5 ml	1002-105
SPIN™ Filters	10	1002-106
Catch Tubes	10	1002-107
Homogenization Matrix/Tubes	5	1002-110

**The* Ancient DNA GLASSMILK®, Dehybernation Solution A, *and* Salton Wash #1 *contain Guanidine Thiocyanate. Use with proper precaution.*

***Add 13.5 ml of 100% Ethanol to contents of Ancient DNA Alcohol Wash bottle and mix before use.*

Two different DeHybernation Solutions are supplied with this kit. DeHyb A is a guanidine based solution, DeHyb B is an aqueous EDTA based solution. Unfortunately with the large diversity of samples and their conditions (degree of preservation and environment) it is difficult to determine which DeHyb solution will be most efficacious. Therefore, preliminary experiments are necessary.

1002-200 (100 preps)

Name	Volume	Catalog #
Dehybernation Solution A*	110 ml	1002-201
Dehybernation Solution B	110 ml	1002-209
Dehyb Solution A_2	18 ml	1002-211
Ancient DNA **GLASSMILK**〉*	35 ml	1002-202
Salton Wash #1*	60 ml	1002-203
Salton Wash #2	60 ml	1002-208
Ancient DNA Alcohol Wash**	15 ml	1002-204
DNA-free Elution Solution	15 ml	1002-205
SPIN™ Filters	100	1002-206
Catch Tubes	100	1002-207
Homogenization Matrix/Tubes	5	1002-110

**The* Ancient DNA GLASSMILK〉, Dehybernation Solution A, *and* Salton Wash #1 *contain Guanidine Thiocyanate. Use with proper precaution.*

***Add contents of Ancient DNA Alcohol Wash (15ml) to 135ml 100% Ethanol.*

Two different DeHybernation Solutions are supplied with this kit. DeHyb A is a guanidine based solution, DeHyb B is an aqueous EDTA based solution. Unfortunately with the large diversity of samples and their conditions (degree of preservation and environment) it is difficult to determine which DeHyb solution will be most efficacious. Therefore, preliminary experiments are necessary.

Introduction

The **GENECLEAN® Kit for Ancient DNA** is designed for isolation of DNA from samples of bone, preserved tissue or animal by-products. The reagents are formulated carefully to prevent contamination by contemporary DNA. Each prep is capable of isolating 20 µg of DNA. The resulting samples are readily amplified by PCR or other methods of amplification.

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

Protocols

For isolation of DNA from non-viable tissues or samples which have historical or forensic value.

Manual Protocol

Incubation with Proteinase K has been reported to increase yields.
see Note, page 9

1. Add 100-500 mg homogenized or powdered sample to **1 ml DeHybernation Solution*τ** in a nucleic acid-free microcentrifuge tube.

2. Incubate at 45-60° C between 2 and 12 hours with mixing (longer incubation times may be necessary).

3. Centrifuge sample at high speed for 5 minutes to pellet particulate material. Transfer supernatant to a new nucleic acid-free microcentrifuge tube. Add **300 µl Ancient DNA GLASSMILK®** suspension. Incubate at room temperature for 10-30 minutes with mixing.

4. Transfer suspension to a **SPIN™ Filter** and **Catch Tube**. Centrifuge at 14,000 x g in microcentrifuge for 1 minute or until liquid is transferred to **Catch Tube**. (Empty **Catch Tube** as needed).

5. Add **0.5 ml Salton Wash #1** and centrifuge at 14,000 x g to clean **GLASSMILK®**/DNA complex.

6. Add **0.5 ml Salton Wash #2** and centrifuge at 14,000 x g to clean **GLASSMILK®**/DNA complex.

7. Add **0.5 ml Ancient DNA Alcohol Wash**** and centrifuge to empty filter of **Wash Solution** . Repeat. [**Important**: Be sure to add alcohol prior to first use**].

8. Empty **Catch Tube** and centrifuge for 2 minutes to "dry" **GLASSMILK®** in **SPIN™ Filter.**

τ There are two different Dehyb solutions supplied with this kit. They differ in composition and each is effective in the isolation of DNA. Because the materials from which the DNA is to be extracted vary so greatly we are not able to recommend one over another.

* There is an additional detergent supplied with this kit that can be used in conjunction with DeHyb A. In certain applications this detergent (DeHyb A2) has resulted in improved DNA yields. If you choose to use DeHyb A2, then the amount of DeHyb A should be decreased to 850 ul, and 150 ul of DeHyb A2 must be added just prior to incubation or homogenization (vortexing or processing in the FastPrep™ FP120 Instrument).

***See pages 3 or 4 for instructions on constituting* Ancient DNA Alcohol Wash.**

9. Place filter into a **DNA-free Elution Catch Tube**. Add **50-100 µl DNA-free Elution Solution**. Resuspend pellet by hand or briefly vortex (1-2 seconds, any longer will result in damage to the filter). Centrifuge for 1 minute to transfer eluate to **Catch Tube**.

Optional: Elute a second time.

10. Remove **SPIN™ Filter** and discard. DNA is ready to use in amplification reaction without further manipulation.

Protocol Using Homogenization Matrix/Tubes

Incubation with Proteinase K has been reported to increase yields.
See Note, page 9.

1b. Add 100-500 mg sample (slightly granular form, whole chunks will not homogenize well) to **1 ml DeHybernation Solution*[τ]** in the **Homogenization Matrix/Tube**.

2b. Vortex for 15 minutes at full speed.

3b. Incubate at 45-60° C between 2 and 12 hours with mixing (longer incubation times may be necessary).

4b. Centrifuge sample at high speed for 5 minutes to pellet particulate material. Transfer supernatant to a new nucleic acid-free tube. Centrifuge again for 3 minutes (to remove any remaining particulates). Transfer supernatant to a new nucleic acid-free tube.

5b. Add **300 µl Ancient DNA GLASSMILK®** suspension. Incubate at room temperature for 10-30 minutes with mixing. Continue with step 4 in manual protocol (page 6).

[τ] There are two different Dehyb solutions supplied with this kit. They differ in composition and each is effective in the isolation of DNA. Because the materials from which the DNA is to be extracted vary so greatly we are not able to recommend one over the other.

*There is an additional detergent supplied with this kit that can be used in conjunction with DeHyb A. In certain applications this detergent (DeHyb A_2) has resulted in improved DNA yields. If you choose to use DeHyb A_2, then the amount of DeHyb A should be decreased to 850 ul, and 150 ul of DeHyb A_2 must be added just prior to homogenization (vortexing).

Protocol for Use with FastPrep™ FP120 Instrument.

Incubation with Proteinase K has been reported to increase yields.
See Note, page 9.

1c. Add 100-500 mg sample (slightly granular form, whole chunks will not homogenize well) to 1 ml of **DNA DeHybernation Solution***$^{\tau}$ in a **2 ml Homogenization Matrix Tube**.

2c. Process at a setting of 6 in **FastPrep**™ Instrument for 5-45 seconds to homogenize sample. Softer samples require shorter times.

3c. Incubate at 45-60° C between 2 and 12 hours with mixing (longer incubation times may be necessary).

4c. Centrifuge sample at high speed for 5 minutes to pellet particulate material. Transfer supernatant to a new nucleic acid-free tube. Centrifuge again for 3 minutes (to remove any remaining particulates). Transfer supernatant to a new nucleic acid-free tube.

5c. Add **300 µl Ancient DNA GLASSMILK®** suspension. Incubate at room temperature for 10-30 minutes with mixing. Continue with step 4 in **manual protocol (page 6).**

$^{\tau}$There are two different Dehyb solutions supplied with this kit. They differ in composition and each is effective in the isolation of DNA. Because the materials from which the DNA is to be extracted vary so greatly we are not able to recommend one over the other.

*There is an additional detergent supplied with this kit that can be used in conjunction with DeHyb A. In certain applications this detergent (DeHyb A_2) has resulted in improved DNA yields. If you choose to use DeHyb A_2, then the amount of DeHyb A should be decreased to 850 ul, and 150 ul of DeHyb A_2 must be added just prior to incubation or homogenization (vortexing or processing in the FastPrep™ Instrument).

Appendix

A prior washing of the DNA/**Ancient DNA GLASSMILK®** pellet with ***300 µl 1:1 solution of acetone:ethanol*** prior to the wash step in step 7 (page 6) will enhance purity of DNA if subsequent reactions are not working properly.

Note

Due to the chemical denaturants contained in both **DeHyb Solutions**, a preincubation with ***Proteinase K*** is suggested. The following protocol was submitted by a customer.

Note: Starting samples are 240-400 mg of powder drilled from bone.

Overnight Soaking Solution

5 µl 0.5 M EDTA
200 µl 10% SDS
200 µl 20 mg/ml Proteinase K

1. Samples were rotated and incubated at 37° C for 12-15 hours.

2. **1 ml DeHybernation Solution A** was added to each sample and rotated for 2-4 hours at 60° C.

3. Samples were spun in centrifuge to pellet particulate.

4. Supernatant was transferred to clean tube and **1.2 ml Ancient DNA GLASSMILK®** and **3.0 ml DeHybernation Solution A** were added.

5. Samples were rotated for 2 hours at 35-40° C.

6. Samples were centrifuged at 4,000 rpm for 1 minute to pellet **Ancient DNA GLASSMILK®**. Supernatant was discarded.

7. **0.5 ml Salton Wash #1** was added to resuspend pellet, which was then transferred to a **SPIN™ Filter**.

8. The protocol, starting with Step 6 (page 6), was followed from this point forward.

Product Use Limitation & Warranty

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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.
PCR* process is covered by U.S. patents owned by Hoffman - La Roche, Inc.

Technical Support and Ordering Information

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