

DNA IQ™ System

Use with Hair Shafts

This document provides a detailed protocol for DNA purification from hair shafts using the DNA IQ™ System and Tissue and Hair Extraction Kit (for use with DNA IQ™). Hair shafts contain little or no nuclear DNA, but they are a rich source of mitochondrial DNA.

I. Product Information

Product	Size	Cat.#
DNA IQ™ System	100 samples	DC6701
	400 samples	DC6700
Tissue and Hair Extraction Kit (for use with DNA IQ™)	100 reactions	DC6740
MagneSphere® Technology Magnetic Separation Stand	2 positions	Z5332
	12 positions	Z5342

II. Protocol

A. Preparation of Reagents

Materials to Be Supplied by the User

- 95–100% ethanol
- isopropyl alcohol
- nuclease-free water
- Tissue and Hair Extraction Kit (for use with DNA IQ™)(Cat.# DC6740)

Preparation of 1X Wash Buffer

Note: The 2X Wash Buffer is supplied with the DNA IQ™ System (Cat.# DC6700 and DC6701).

1. For Cat.# DC6701 (100 samples), add 15ml of 95–100% ethanol and 15ml of isopropyl alcohol to the 2X Wash Buffer.

For Cat.# DC6700 (400 samples), add 35ml of 95–100% ethanol and 35ml of isopropyl alcohol to the 2X Wash Buffer.

2. Replace cap, and mix by inverting several times.
3. Mark label to record the addition of alcohols. Label bottle as 1X Wash Buffer. Solution can be stored at room temperature. Make sure the bottle is closed tightly to prevent evaporation.

Preparation of 1M DTT

Note: The DTT is supplied with the Tissue and Hair Extraction Kit (Cat.# DC6740).

Dissolve 5g of DTT in nuclease-free water so that the final volume is 32.4ml. The final concentration of DTT will be 1M. Dispense the DTT into smaller aliquots that reflect usage, and store at –20°C.

Preparation of Lysis Buffer

1. Determine the total amount of Lysis Buffer to be used. DNA isolation from hair shafts requires 450µl of Lysis Buffer per sample.
2. Add 1µl of 1M DTT for every 100µl of Lysis Buffer.
3. Mix by inverting several times.
4. Mark and date label to record the addition of DTT. This solution can be stored at room temperature for up to one month if sealed.

Preparation of Stock Proteinase K Solution

The Proteinase K and Incubation Buffer are supplied with the Tissue and Hair Extraction Kit (Cat.# DC6740).

1. Add 5.5ml of Incubation Buffer to the bottle of lyophilized Proteinase K, and gently swirl to dissolve. The final concentration of proteinase K will be 18mg/ml.
2. Dispense the stock Proteinase K solution into smaller aliquots that reflect usage, and store at –20°C for up to 1 year. Proteinase K can be frozen and thawed up to five times with no significant loss in activity. Prior to use, Proteinase K should be thawed and stored on ice.



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Continued

B. DNA Purification From Hair Shafts

1. Cut one or more 1–4cm section of hair shaft, and place in a 1.5ml microcentrifuge tube.
2. Add 100µl of 1M DTT and 75µl of the stock Proteinase K solution. Make sure the entire hair shaft is covered. Mix, and incubate at 56°C for 1 hour.

Note: The hair shaft may remain intact but will dissolve when the Lysis Buffer is added in the next step.

3. Place the sample at room temperature. Add 2 volumes (350µl) of prepared Lysis Buffer and 7µl of resuspended DNA IQ™ Resin from the DNA IQ™ System. Vortex for 3 seconds at high speed and incubate for 5 minutes at room temperature. Vortex for 3 seconds every 1 minute during this 5-minute incubation.

Note: If visible pieces of hair remain after incubation in the Lysis Buffer, the pieces can be removed, the solution can be transferred to another tube or the pieces can be left in the tube, as they will not interfere with DNA purification.

4. Vortex for 2 seconds at high speed. Place tube in the magnetic stand. Separation will occur instantly.

Note: If the resin does not form a distinct pellet on the side of the tube, vortex the tube and quickly place it back in the stand.

5. Carefully remove and discard all of the solution without disturbing the resin pellet at the side of the tube.

6. Add 100µl of prepared Lysis Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.

7. Return tube to the magnetic stand. Carefully remove and discard all Lysis Buffer.

8. Add 100µl of prepared 1X Wash Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.

9. Return tube to the magnetic stand. Dispose of all Wash Buffer.

10. Repeat Steps 8 and 9 two more times for a total of three washes. Make sure all of the solution has been removed after the last wash.

11. With the tube in the magnetic stand and the lid open, air-dry the resin for 5 minutes. Do not dry for more than 20 minutes, as this may inhibit elution of DNA.

12. Add 25µl of Elution Buffer from the DNA IQ™ System.

Note: The volume of Elution Buffer can be adjusted to provide an appropriate range of DNA concentrations.

13. Close the lid, and vortex tube for 2 seconds at high speed. Incubate at 65°C for 5 minutes.

14. Remove the tube from the heat source, and vortex for 2 seconds at high speed. Immediately place the tube on the magnetic stand.

15. Carefully transfer the DNA solution to a container of choice.

Note: The DNA solution can be stored at 4°C for short-term storage or at –20°C or –70°C for long-term storage.

