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# Hair Mitochondrial DNA Isolation Kit — Product Insert

**Product# 69400**

Over the past several decades, mitochondrial DNA (mtDNA) has played a role in forensic analysis of various criminal cases. A few hairs left at a crime scene contain enough mtDNA for extraction. The hair shaft, which protrudes out of the scalp, does not contain any nuclear DNA. It does, however, contain mtDNA. While nuclear DNA is present in only two copies per cell, the small circular mtDNA molecule is present in hundreds to thousands of copies per cell making it very abundant. Mitochondrial DNA is maternally inherited, and all of a woman's offspring will have the same mtDNA profile. An advantage of this is that a single maternal relative of that person may provide a reference sample for comparison to a sample found at a crime scene.

Norgen's Hair Mitochondrial DNA Isolation Kit provides a fast, reliable and simple procedure for isolating mtDNA from hair shafts. Purification is based on spin column chromatography and the DNA is preferentially purified from other components. Typical yields will vary depending on the sample input volume used. The purified DNA is compatible with all downstream applications including PCR and NGS.

**Norgen's Purification Technology**

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The process provides a simple and convenient mitochondrial DNA isolation protocol for hair samples. First, DTT, Proteinase K and Lysis Additive A are added to the hair shaft and the mixture is incubated at 55˚C for 30 minutes. Once the hair shaft is completely dissolved any undigested hair is removed by centrifugation. Then clean supernatant is collected and isopropanol and Lysis Buffer B are added. The lysate is then loaded onto a spin-column. Norgen's spin column binds nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while the proteins and other contaminants are removed in the flowthrough or retained on top of the resin. The bound DNA is then washed using the provided Wash Solution A, and the purified DNA is eluted using the Elution Buffer B. The purified mtDNA (and genomic DNA if hair root is used) is free of all inhibitors and can be used in sensitive downstream applications including PCR and sequencing.

**Kit Components**

| Component | Product #69400 (50 Preps) |
|---|---|
| DTT | 6 mL |
| Proteinase K | 4 mL |
| Lysis Additive A | 6 mL |
| Lysis Buffer B | 20 mL |
| Wash Solution A | 38 mL |
| Elution Buffer B | 8 mL |
| Micro Spin Columns | 50 |
| Elution Tubes | 50 |
| Collection Tubes | 50 |
| Product Insert | 1 |

**Advantages**

- Fast and easy processing using rapid spin-column format
- Isolate high quality mitochondrial DNA
- Recovered DNA is compatible with various downstream applications

**Storage Conditions and Product Stability**

Store DTT at -20°C upon arrival. All other solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers. The kit contains a ready to-use Proteinase K solution, which is dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 2 years after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

**General Precautions and Disclaimers**

This kit is designed for research purposes only. It is not intended for human or diagnostic use. All necessary precautions recommended by the appropriate authorities in the country of use should be taken.

**Product Warranty and Satisfaction Guarantee**

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

**Safety Information**

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

**Lysis Buffer B** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

**CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.**

**Customer-Supplied Reagents and Equipment**

- Benchtop microcentrifuge (> 14,000 RPM)
- 1.5 mL or 2 mL Nuclease-free microcentrifuge tubes
- Isopropanol (2-propanol)
- Vortex mixer
- Micropipettors and multichannel pipettes
- 55 °C incubator

# Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

**Notes Prior to Use**

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Preheat a water bath or heating block to 55ºC.
- Prepare a working concentration of the Wash Solution A by adding 90 mL of 96 - 100% ethanol (provided by the user) to each supplied bottle containing the concentrated Wash Solution A. This will give a final volume of 128 mL. The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.
- Always vortex proteinase K before use.
- Dispense the DTT into smaller aliquots that reflect usage, and store at –20°C.

**1. Lysate Preparation**

a. Obtain 9-10 1 cm pieces of hair shaft and place in a clean microcentrifuge tube.
b. Add 100 µL of **DTT** to the tube.
c. Add 70 µL of **Proteinase K** (vortex before use).
d. Add 60 µL of **Lysis Additive A.**
e. Mix briefly by inversion and ensure the hair shafts return to the bottom of the tube and are submerged in liquid.
f. Incubate the mixture for 30 minutes at 55°C. Every 10 minutes briefly spin down the hair shafts to the bottom of the tube.
g. Centrifuge for 1 minute at 14,000 RPM and take clean supernatant to a clean micro tube.
h. Add 250 µL of **Isopropanol** and vortex for 10 seconds to mix.
i. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
j. Add 250 µL of **Lysis Buffer B**. Vortex to mix, and then briefly spin again.

**2. Binding to Column**

a. Assemble a column with one of the provided collection tubes.
b. Apply up to 600 µL of the lysate to the Micro Spin column and centrifuge for 1 minute at 10,00 RPM

**Note:** Ensure that all of the lysate has passed through into the collection tube. If the entire lysate volume has not passed, centrifuge for an additional 1 minute at 14,000 RPM.

c. Discard the flow through and reassemble the Micro Spin column with its collection tube.
d. Repeat steps **2b and 2c** for the rest of the lysate.

**3. Column Wash**

a. Apply 500 µL of **Wash Solution A** (ensure ethanol was added) to the column and centrifuge for 1 minute at 10,000 RPM.
b. Discard the flow through and reassemble the Micro Spin column with its collection tube.
c. Repeat steps **3a and 3b** for an additional wash.
d. Spin the Micro Spin column for 2 minutes in order to thoroughly dry the column at 14,000 RPM for 2 minutes.
e. Discard the collection tube.

**4. DNA Elution**
a. Place the Micro Spin column into a provided 1.7 mL elution tube.
b. Add 30-50 µL of **Elution Buffer B** and incubate for 1 minute at room temperature.
c. Centrifuge for 1 minute at 10,000 RPM to elute the DNA.

**5. Storage of DNA**
The purified DNA sample may be stored at 4 °C for a few days. It is recommended that samples be placed at -20C for long term storage.

## Troubleshooting Guide

| Problem | Possible Cause | Solution and Explanation |
|---|---|---|
| Poor DNA Recovery | Incomplete lysis of hair shaft | Ensure that the hair shaft was completely dissolved in the mixture of DTT, Lysis Additive A and Proteinase K. |
| | Column become clogged | Do not exceed the recommended amounts of starting materials. Also ensure that any undigested hair shaft is removed by centrifugation to collect only clean supernatant |
| | An alternative elution solution was used | It is recommended that the Elution Buffer B supplied with this kit be used for maximum DNA recovery. |
| | Isopropanol was not added to the clean lysate | Ensure that the appropriate amount of Isopropanol is added to the lysate before binding to the column. |
| | Ethanol was not added to the Wash Solution A | Ensure that 90 mL of 96-100% ethanol is added to the supplied Wash Solution A prior to use. |
| DNA does not perform well in downstream applications | DNA was not washed 3 times with the provided Solution A | Traces of salt from the binding step may remain in the sample if the plate is not washed 3 times with Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column. |
| | Ethanol carryover | Ensure that the dry vacuum or dry spin under the DNA Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications. |

| Related Products | Product # |
|---|---|
| Mitochondrial DNA Hypervariable Region I and II PCR Amplification Kit | 52800 |

**Technical Assistance**

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Hair Mitochondrial DNA Isolation Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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