



**Promega**

Technical Bulletin

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# **Tissue and Hair Extraction Kit (for use with DNA IQ™ ) Protocol**

INSTRUCTIONS FOR USE OF PRODUCT DC6740.



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# Tissue and Hair Extraction Kit (for use with DNA IQ™ ) Protocol

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## 1. Description

The DNA IQ™ System<sup>(a)</sup>, which uses a novel paramagnetic resin to purify DNA, contains a strong denaturing agent that disrupts many sample types in preparation for DNA purification. However, some samples such as tissue and hair are not sufficiently disrupted with the DNA IQ™ Lysis Buffer and thus require pretreatment with proteinase K to ensure sample lysis. The reagents and protocols provided with the Tissue and Hair Extraction Kit (for use with DNA IQ™) are sufficient to break up most tissue and hair samples and remove proteins and other components from the DNA. The DNA can then be purified using the DNA IQ™ System.

### Genomic and Mitochondrial DNA

When used together, the Tissue and Hair Extraction Kit (for use with DNA IQ™) and the DNA IQ™ System are designed to extract and purify double-stranded or single-stranded genomic and mitochondrial DNA. If the sample is contaminated with microbes, the microbial DNA will also be isolated. DNA molecules less than 80 bases in length, which act as PCR inhibitors, are selectively removed to provide DNA that is more efficiently amplified.

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## Increased Yields with the Tissue and Hair Extraction Kit (for use with DNA IQ™)

When working with reference samples, the DNA IQ™ System is designed to isolate a consistent amount of DNA following the *DNA IQ™ System – Database Protocol* (Technical Bulletin #TB297). This consistency depends upon saturation of the DNA IQ™ Resin by an excess of DNA, and the system has been calibrated using a variety of biological materials. When using the Tissue and Hair Extraction Kit (for use with DNA IQ™) with the DNA IQ™ System, the proteinase K digestion eliminates material that competes for binding sites on the DNA IQ™ Resin. As a result, the proteinase K pretreatment improves yield from certain samples. Different sample types may be affected differently by proteinase K treatment, so to take advantage of the quantitative aspects of the DNA IQ™ System, prior optimization will need to be done with each sample type.

### Hair

Hair is a commonly found sample at crime scenes, but processing of nuclear DNA from hair is frequently unsuccessful. This is primarily due to the life cycle of hair. As the hair ages and prepares to fall out, the nucleus and DNA content are degraded (1). Many shed hairs contain little useful nuclear DNA, and the DNA IQ™ System cannot overcome this fact. The DNA IQ™ System does eliminate very small fragments of DNA, which can act as PCR inhibitors, and thus amplification results from limited amounts of template DNA may be improved. In cases where the DNA yield is poor or the DNA is degraded, results may also be improved through the amplification of smaller DNA targets (2).

DNA purification from unwashed hair samples may help increase DNA yield, as surrounding cells are frequently attached to shed hairs. However, contamination may be observed. While many plucked hairs will supply enough DNA from a single hair using the DNA IQ™ Lysis Buffer without a proteinase K treatment, the addition of a proteinase K treatment can significantly increase DNA yield. A small number of individuals have proteinase K-resistant hair that lyses well only in the presence of high concentrations of DTT. The Incubation Buffer/Proteinase K solution of the Tissue and Hair Extraction Kit (for use with DNA IQ™) contains a final concentration of 100mM DTT to help lyse these resistant hair samples.

### Mitochondrial DNA

While hair shafts contain no significant nuclear DNA, they are a rich source of mitochondrial DNA. Proteinase K treatment in the presence of high concentrations of DTT is sufficient to partially disrupt the hair shaft so that the DNA IQ™ Lysis Buffer is able to extract the majority of the mitochondrial DNA. The DNA IQ™ Resin is very effective at removing hair pigments that inhibit PCR amplification.

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## Tissue

The reagents and protocol supplied with the Tissue and Hair Extraction Kit (for use with DNA IQ™) allow DNA purification from a large variety of tissue types when combined with the DNA IQ™ System. As noted above, the quantitative features of the DNA IQ™ System will be affected by proteinase K digestion, and if desired, yields should be calibrated. The ability to obtain DNA from samples depends on the quality of the starting materials. Tissue that has been exposed to high temperatures for a considerable period of time may yield severely damaged DNA that may not amplify well. DNA can be extracted more efficiently if the tissue is finely minced prior to proteinase K treatment.

## Formalin-Fixed Tissue

Formalin cross-links DNA and protein and thus presents a special problem in obtaining DNA for amplification purposes. The use of the Tissue and Hair Extraction Kit (for use with DNA IQ™) in conjunction with the DNA IQ™ System is very effective at obtaining DNA from both large pieces and thin sections of formalin-fixed, paraffin-embedded tissue. However, tissue that has been stored in formalin for an extensive period of time will be more highly cross-linked, and the DNA purified from such samples may not allow efficient amplification, especially of larger amplicons. Increasing the extension time during amplification may help to balance yields between small and large amplification products.

## Bone

Bone needs to be decalcified and large volumes of proteinase K are needed for DNA purification. To purify DNA from bone samples, the Bone Incubation Buffer, Proteinase K, Incubation Buffer, DTT and the DNA IQ™ System are required. Additional Lysis Buffer may be required, depending on sample mass and sample number. Please contact Promega Technical Services ([genetic@promega.com](mailto:genetic@promega.com)) for information about the Bone Incubation Buffer and available protocols for using the DNA IQ™ System for DNA purification from bone.

## 2. Product Components and Storage Conditions

<u>Product</u>	<u>Size</u>	<u>Cat.#</u>
Tissue and Hair Extraction Kit (for use with DNA IQ™)	100 reactions	DC6740

The kit includes sufficient reagents for 100 reactions using hair follicles or fresh, frozen or formalin-fixed, paraffin-embedded tissue or 75 reactions using hair shafts. Includes:

- 50ml Incubation Buffer
- 100mg Proteinase K
- 5g DTT
- 1 Protocol

**Storage Conditions:** Store Proteinase K and DTT at -20°C. The Incubation Buffer can be stored at -20°C to 25°C.

## 3. Protocol for Use of the Tissue and Hair Extraction Kit (for use with DNA IQ™) With the DNA IQ™ System

### Materials to Be Supplied by the User

- DNA IQ™ System (Cat.# DC6700 or DC6701)
- 56°C heat block, water bath or thermal cycler
- aerosol-resistant micropipette tips
- nuclease-free water
- MagneSphere® Technology Magnetic Separation Stand (twelve-position) (Cat.# Z5342) or MagneSphere® Technology Magnetic Separation Stand (two-position) (Cat.# Z5332)
- 95-100% ethanol
- isopropyl alcohol
- 65°C heat block, water bath, or thermal cycler
- vortex mixer
- Microtubes, 1.5ml (Cat.# V1231)

 Use of gloves and aerosol-resistant micropipette tips is highly recommended to prevent cross-contamination.

### 3.A. Preparation of Reagents

#### Preparation of Stock Proteinase K Solution

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. The Proteinase K can be frozen and thawed up to 5 times with no significant loss in activity. Prior to use, Proteinase K should be thawed and stored on ice.

### Preparation of 1M DTT

1. 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 Incubation Buffer/Proteinase K Solution

The Incubation Buffer/Proteinase K Solution should be prepared fresh for each set of DNA purifications.

1. Prepare the Incubation Buffer/Proteinase K solution by combining the Incubation Buffer, 1M DTT and the stock Proteinase K solution in the proportions indicated below. Prepare 25–100µl of Incubation Buffer/Proteinase K solution for each hair follicle sample (Section 3.B). Prepare 50–100µl of Incubation Buffer/Proteinase K solution for each tissue sample (Section 3.C). The final concentration of Proteinase K will be 1.8mg/ml.

Component	Volume
Incubation Buffer	800µl
1M DTT	100µl
stock Proteinase K solution	100µl
<b>total volume</b>	<b>1,000µl</b>

2. Mix gently, and store on ice during use.

### Preparation of 1X Wash Buffer

The 2X Wash Buffer used to prepare the 1X Wash Buffer is supplied with the DNA IQ™ System (Cat.# DC6700 and DC6701), which must be purchased separately to use the following protocols. The 2X Wash Buffer is not supplied with the Tissue and Hair Extraction Kit (for use with DNA IQ™).

1. For the DNA IQ™ System (Cat.# DC6701: 100 samples) add 15ml of 95–100% ethanol and 15ml of isopropyl alcohol to the 2X Wash Buffer.  
  
For the DNA IQ™ System (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 bottle is closed tightly to prevent evaporation.

### Preparation of Lysis Buffer

The Lysis Buffer is supplied with the DNA IQ™ System (Cat.# DC6700 and DC6701), which must be purchased separately in order to use the following protocols. The Lysis Buffer is not supplied with the Tissue and Hair Extraction Kit (for use with DNA IQ™).

1. Determine the total amount of prepared Lysis Buffer to be used. Genomic DNA isolation from hair follicles will require 150–300µl of prepared Lysis Buffer per sample (Section 3.B). Genomic DNA isolation from fresh, frozen or formalin-fixed, paraffin-embedded tissues will require 150–300µl of prepared Lysis Buffer per sample (Section 3.C). Mitochondrial DNA isolation from hair shafts will require 450µl of prepared 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 a month if sealed.

### 3.B. DNA Purification From Hair Follicles and Hair Shafts

1. For genomic DNA, cut the hair follicle from the hair shaft, and place in a 1.5ml tube. Add 25–100µl of freshly prepared Incubation Buffer/Proteinase K solution (enough to cover entire sample). Mix, close the tube cap and incubate at 56°C for 1 hour.

For mitochondrial DNA, cut one or more 1–4cm sections of hair shaft, and place in a 1.5ml tube. Add 100µl of 1M DTT and 75µl of the stock Proteinase K solution. Mix, close the tube cap and incubate at 56°C for 1 hour. Make sure the entire hair shaft is covered.

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


2. Place the sample at room temperature, and add 2 volumes of 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.

**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.

3. Vortex for 2 seconds at high speed. Place tube in the MagneSphere® Technology Magnetic Separation Stand. Separation will occur instantly.

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

4. Carefully remove and dispose of all solution without disturbing the resin pellet on the side of the tube.

5. Add 100µl of prepared Lysis Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.
  6. Return tube to the magnetic stand. Carefully remove and discard all Lysis Buffer.
  7. Add 100µl of prepared 1X Wash Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.
  8. Return tube to the magnetic stand, and discard all Wash Buffer.
  9. Repeat Steps 7 and 8 two more times for a total of three washes. Make sure all of the solution has been removed after the last wash.
  10. 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 removal of DNA.
11. Add 25–100µl of Elution Buffer, depending on the quantity of biological material used. A lower elution volume ensures a higher final concentration of DNA.
  12. Close the lid, and vortex tube for 2 seconds at high speed. Incubate at 65°C for 5 minutes.
  13. Remove the tube from the heat source, and vortex for 2 seconds at high speed. Immediately place on the magnetic stand.
  14. 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.

### 3.C. DNA Purification From Fresh, Frozen or Formalin-Fixed, Paraffin-Embedded Tissue

1. Place approximately 1mg of tissue or less in a 1.5ml microcentrifuge tube. The amount of tissue is based on the condition and type of sample. Larger amounts of sample, up to 25mg of tissue, can be used if tissue is minced prior to proteinase K treatment.

**Notes:**

1. Thin sections of formalin-fixed, paraffin-embedded tissue do not need to be deparaffinized or minced, as most of the tissue is accessible to proteinase K.
2. Large amounts of tissue will not digest well and may contain large quantities of DNA, making the lysate very viscous and impeding collection of the DNA IQ™ Resin during the magnetic separation steps.

### 3.C. DNA Purification From Fresh, Frozen or Formalin-Fixed, Paraffin-Embedded Tissue (continued)

2. Add 50–100 $\mu$ l of freshly prepared Incubation Buffer/Proteinase K solution, and incubate at 56°C for 2 hours to overnight depending on sample type. Most tissues will be digested completely in 2 hours, but formalin-fixed, paraffin-embedded tissue should be incubated overnight.

**Note:** Add enough Incubation Buffer/Proteinase K solution to cover the entire sample.

3. Remove sample from the heat source, and add 2 volumes of prepared Lysis Buffer.

**Notes:**

1. If large amounts of paraffin are present in the sample, a paraffin layer may form at the top of the tube. Transfer the DNA solution to a fresh tube, being careful to avoid the paraffin layer, before adding the 2 volumes of Lysis Buffer.
2. If the tissue does not dissolve completely in the Lysis Buffer, the solution can be placed in a new tube. Large, undigested pieces of tissue should not be transferred to the new tube. Small pieces of undigested tissue can be removed by centrifugation, and the soluble material can be transferred to a new tube. A small amount of undigested tissue will not interfere with the DNA purification.
4. Add 7 $\mu$ l 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.
5. Vortex for 2 seconds at high speed. Place tube in the MagneSphere® Technology Magnetic Separation Stand. Separation will occur instantly.  
**Note:** If resin does not form a distinct pellet on the side of the tube, vortex the tube and quickly place back in the stand.
6. Carefully remove and discard all of the solution without disturbing the resin pellet on the side of the tube.
7. Add 100 $\mu$ l of prepared Lysis Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.
8. Return tube to the magnetic stand, and discard all Lysis Buffer.
9. Add 100 $\mu$ l of prepared 1X Wash Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.
10. Return tube to the magnetic stand. Discard all Wash Buffer.
11. Repeat Steps 9 and 10 two more times for a total of three washes. Make sure all of the solution has been removed after the last wash.
12. 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 removal of DNA.

13. Add 25–100µl of Elution Buffer, depending on the quantity of biological material used. Lower elution volume ensures a higher final concentration of DNA.
14. Close the lid, and vortex tube for 2 seconds at high speed. Incubate at 65°C for 5 minutes.
15. Remove the tube from the heat source, and vortex for 2 seconds at high speed. Immediately place on the magnetic stand.
16. 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.

#### 4. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or distributor. Contact information available at: [www.promega.com](http://www.promega.com). E-mail: [genetic@promega.com](mailto:genetic@promega.com)

<u>Symptoms</u>	<u>Causes and Comments</u>
Sample does not digest	<p>Tissue pieces are too large. Mince tissue into smaller pieces.</p> <hr/> <p>DTT was not added to Incubation Buffer/ Proteinase K solution. Be sure Incubation Buffer/Proteinase K solution contains a final concentration of 100mM DTT (see Section 3.A).</p>
Poor DNA yield	<p>Too much protein is present in starting material. Use less sample. Large amounts of protein in the sample will compete with DNA for binding to the DNA IQ™ Resin.</p> <hr/> <p>Too much microbial DNA. Microbial DNA will compete with human DNA for binding to the DNA IQ™ Resin.</p> <ul style="list-style-type: none"> <li>• Collect sample from the least contaminated area of tissue.</li> <li>• Increase the amount of resin to 20µl to increase overall yield of total DNA.</li> </ul>
Poor amplification of large amplification products	<p>Too much DNA in the amplification. Quantitate human-specific DNA.</p> <hr/> <p>DNA in sample is compromised. Double the extension time of each amplification cycle to compensate for damaged or degraded DNA.</p>

## 5. References

1. Linch, C.A., Smith, S.L. and Prahlow, J.A. (1998) Evaluation of the human hair root for DNA typing subsequent to microscopic comparison. *J. Forensic Sci.* **43**, 305-14.
2. Hellmann, A. *et al.* (2001) STR typing of human telogen hairs—A new approach. *Int. J. Legal Med.* **114**, 269-73.

## 6. Related Products

Product	Size	Cat.#
DNA IQ™ System	100 reactions	DC6701
	400 reactions	DC6700
MagneSphere® Technology Magnetic Separation Stand (two-position)	1.5ml	Z5332
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	1.5ml	Z5342
PolyATtract® System 1000 Magnetic Separation Stand	1 each	Z5410
DNA IQ™ Spin Baskets**	1,000/bag	V1221
Microtubes, 1.5ml	1,000/bag	V1231
ART® 20P, Pipet Tip, 20µl	960/pk	DY1071
ART® 200, Pipet Tip, 200µl	960/pk	DY1121
ART® 1000E, Pipet Tip, 1,000µl	800/pk	DY1131
PowerPlex® 16 System**	100 reactions	DC6531
	400 reactions	DC6530
PowerPlex® 1.1 and 2.1 Systems**	100 reactions	DC6501
	400 reactions	DC6500
PowerPlex® 1.2 System**	100 reactions	DC6101

\*\*Not For Medical Diagnostic Use.

<sup>(a)</sup>U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Australian Pat. No. 732756, European Pat. Nos. 0 895 546 and 1 204 741 and Mexican Pat. No. 209436 have been issued to Promega Corporation for methods of isolating biological target materials using silica magnetic particles and simultaneous isolation and quantitation of DNA. Other patents are pending.

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