

DNA IQ™ System

Use with Liquid Blood

The following document gives a detailed protocol for the use of the DNA IQ™ System with liquid blood.

I. Product Information

Product	Size	Cat.#
DNA IQ™ System	100 samples	DC6701
	400 samples	DC6700
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
- 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 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 liquid blood requires 295–325µl of Lysis Buffer per sample depending on volume of blood being processed (Table 1). Make extra Lysis Buffer to allow for loss during pipetting.

Table 1. Total Volume of DNA IQ™ Lysis Buffer Required to Process Given Volume of Liquid Blood

Volume of Blood	Total Volume of DNA IQ™ Lysis Buffer
10µl	295µl
15µl	305µl
20µl	315µl
25µl	325µl

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.

Preparation of Incubation Buffer/Proteinase K Solution

The Incubation Buffer is supplied with the Tissue and Hair Extraction Kit (Cat.# DC6740). The Incubation Buffer/Proteinase K Solution should be prepared fresh for each set of DNA purifications.

Determine the total volume of Incubation Buffer/Proteinase K Solution from the Tissue and

DNA IQ™ System

Use with Liquid Blood

Continued

Hair Extraction Kit (Cat.# DC6740) to be used. DNA isolation from liquid blood requires 80µl of Incubation Buffer/Proteinase K Solution per sample. Make extra to allow for losses during pipetting.

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. The final concentration of proteinase K will be 1.8mg/ml.

Incubation Buffer	800µl
1M DTT	100µl
Stock Proteinase K solution	100µl

Total volume **1,000µl**

2. Mix gently and store on ice during use.

B. DNA Purification From Liquid Blood

Recommended volume of liquid blood for extraction is 10–25µl.

1. Vortex the DNA IQ™ Resin bottle for 10 seconds at high speed or until resin is thoroughly mixed before removing the desired volume of resin to add to the Lysis Buffer. Prepare a stock solution of DNA IQ™ Resin and Lysis Buffer using 7µl of resin and the volume of prepared Lysis Buffer indicated in Table 2. Make extra to allow for losses during pipetting.

Table 2. Volumes of DNA IQ™ Resin and DNA IQ™ Lysis Buffer per Unit Volume of Liquid Blood

Volume of Blood	Volume of DNA IQ™ Resin	Two Volumes of DNA IQ Lysis Buffer
10µl	7µl	195µl
15µl	7µl	205µl
20µl	7µl	215µl
25µl	7µl	225µl

2. Mix liquid blood gently, and place 15µl into a 1.5ml microcentrifuge tube (10–25µl of blood can be used routinely).
3. Add 80µl of Incubation Buffer/Proteinase K Solution to each liquid blood sample. Incubate at 56°C for 1 hour.
4. Vortex the resin/Lysis Buffer mixture for 2 seconds at high speed to ensure suspension of the resin. Add the appropriate volume of the mixture: 202µl for 10µl of liquid blood, 212µl for 15µl of liquid blood, 222µl for 20µl of liquid blood, 232µl for 25µl of liquid blood. The resin/Lysis Buffer mixture should be mixed again if resin begins to settle while dispensing aliquots.

Note: The amount of resin recommended can capture a maximum of approximately 100ng of DNA. However, yield can vary depending on the individual whose blood is being processed and the amount of blood used.

5. Vortex the sample/Lysis Buffer/resin mixture for 3 seconds at high speed. Incubate for 5 minutes at room temperature. Vortex for 3 seconds every 1 minute during this 5-minute incubation.
6. 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.
7. Carefully remove and discard all of the solution without disturbing the resin pellet at the side of the tube.
8. Add 100µl of prepared Lysis Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.
9. Return tube to the magnetic stand. Carefully remove and discard all Lysis Buffer.
10. Add 100µl of prepared 1X Wash Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.
11. Return tube to the magnetic stand. Dispose of all Wash Buffer.
12. Repeat Steps 10 and 11 two more times for a total of three washes. Make sure all of the solution has been removed after the last wash.
13. 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.
14. Add 100µl of Elution Buffer.
Note: The volume of Elution Buffer can be adjusted to provide an appropriate range of DNA concentrations.
15. Close the lid, and vortex tube for 2 seconds at high speed. Incubate at 65°C for 5 minutes.
16. Remove the tube from the heat source, and vortex for 2 seconds at high speed. Immediately place the tube on the magnetic stand.
17. 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.