



Promega

Technical Bulletin

DNA IQ™ System – Database Protocol

INSTRUCTIONS FOR USE OF PRODUCTS DC6700 AND DC6701.



www.promega.com

DNA IQ™ System – Database Protocol

All technical literature is available on the Internet at: www.promega.com/tbs
Please visit the web site to verify that you are using the most current version of this
Technical Bulletin. Please contact Promega Technical Services if you have questions on use
of this system. E-mail: genetic@promega.com.

1. Description.....	1
2. Product Components and Storage Conditions	3
3. Protocol for the DNA IQ™ System.....	6
A. Preparation of Reagents	6
B. DNA Isolation from Stains and Buccal Swabs.....	8
C. DNA Isolation from Liquid Blood.....	9
4. Troubleshooting.....	11
5. References	12
6. Composition of Buffers and Solutions	12
7. Related Products	13

1. Description

The development of human DNA databases is playing an increasingly important role in the forensic field. To this end, robust commercial STR-based systems have been developed for the analysis of standardized sets of loci. These systems can analyze sub-nanogram amounts of DNA but are limited to a well defined range of DNA. Thus, DNA must be purified, then quantitated accurately to reduce the risk of artifacts.

Two approaches have been used to purify DNA from blood for databasing purposes where sample quantities and nonhuman DNA contamination are not a concern. The first approach is to purify, then quantitate DNA using absorbance measurements or a DNA-specific dye. This approach requires an additional series of steps and is sensitive to the quality of DNA. The second approach is to deliver DNA from a set volume of blood usually attached to a solid support such as FTA® or S&S 903 paper. This approach yields variations in DNA content due to variations in white cell counts or variations on the filter due to sample wicking and differential drying. Additionally, the DNA is bound to the filters and must be removed for quantitation purposes. The high capacity of FTA® paper requires modification of the amplification protocol to prevent excessive amplification and allele imbalances.

The DNA IQ™ System^(a) uses a novel approach for DNA isolation. A paramagnetic resin is used to capture a consistent amount of DNA (Figure 1). The resin has a defined DNA-binding capacity in the presence of excess DNA and will only bind a certain amount of DNA. Yields will be consistent within a single sample type but will differ with different sample types. The typical yield for FTA® blood-card punches is 50-100ng, for liquid blood, 50-200ng; and for buccal swabs, 100-500ng, as determined using the the Quant-iT™ PicoGreen® dsDNA reagent (liquid blood) or Quant-iT™ OliGreen® ssDNA Assay Kit (buccal swabs and FTA® blood-card punches). Since recovery depends on the sampling method, solid support and sample type, laboratories will need to determine the average yield for a single sample type. Once this average yield has been determined, the researcher can bypass the quantitation step typically necessary with other purification procedures.

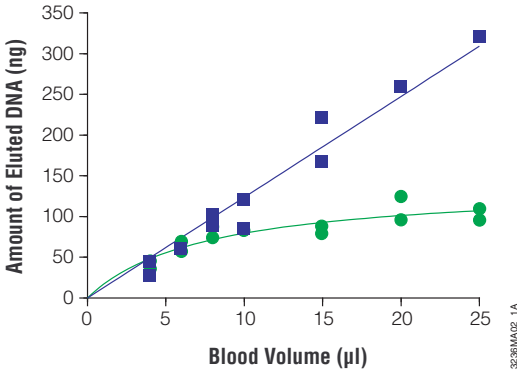


Figure 1. Effect of sample volume on the amount of DNA isolated. DNA was isolated from increasing volumes of liquid blood using a high-capacity DNA-binding resin or the DNA IQ™ Resin. The high-capacity resin shows a linear relationship between eluted DNA and the volume (µl) of blood processed (represented by squares). In contrast, the DNA IQ™ Resin is saturated using the blood volumes tested and thus gives approximately the same amount of DNA regardless of sample size (represented by circles).

The DNA IQ™ System avoids the use of harmful organic solvents such as phenol and eliminates multiple centrifugation steps used in some DNA purification procedures. The DNA IQ™ System procedure is performed using a few simple steps (Figures 2 and 3):

- Extraction of sample (FTA® paper, S&S 903 paper, cotton swab) or lysis of sample
- DNA capture using resin
- Washing of resin
- Elution of DNA from resin

The DNA IQ™ System has been automated using the Beckman Coulter Biomek® 2000 and 3000 Laboratory Automation Workstations and Tecan Freedom EVO® 100 liquid handler. For more information, see the DNA IQ™ System product profile at: www.promega.com/applications/hmnid/productprofiles/automation/. For more information about implementing these methods, contact Promega Technical Services (genetic@promega.com).

2. Product Components and Storage Conditions

Product	Size	Cat.#
DNA IQ™ System	400 samples	DC6700

For Laboratory Use. This system includes:

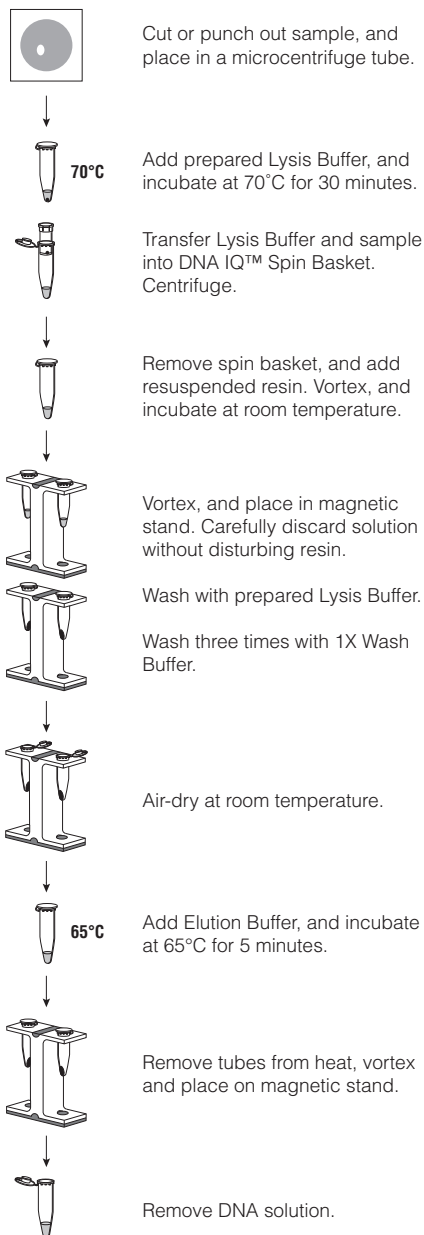
- 3ml Resin
- 150ml Lysis Buffer
- 70ml 2X Wash Buffer
- 50ml Elution Buffer

Product	Size	Cat.#
DNA IQ™ System	100 samples	DC6701

For Laboratory Use. This system includes:

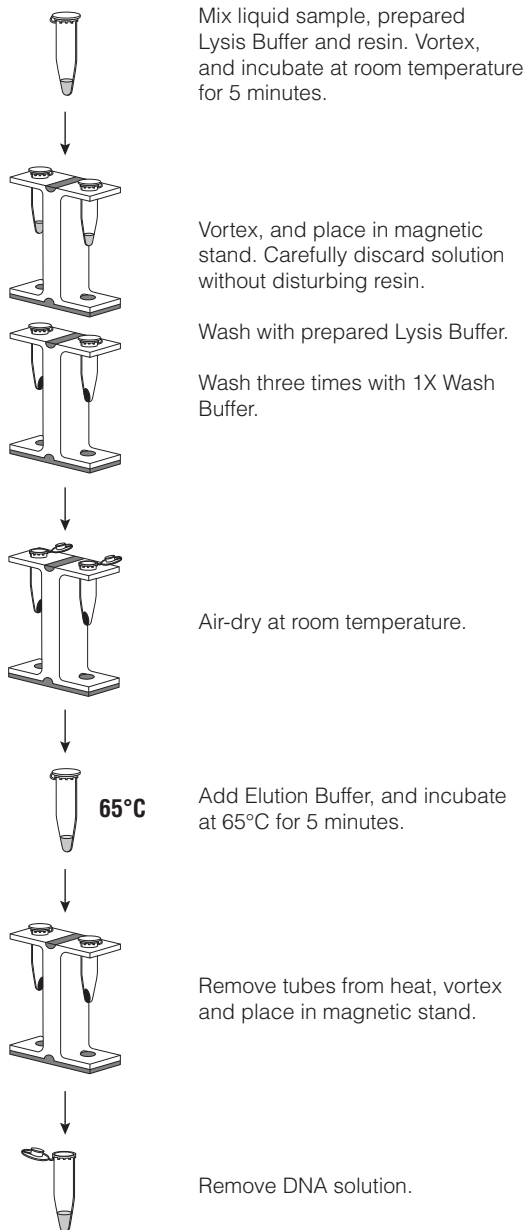
- 0.9ml Resin
- 40ml Lysis Buffer
- 30ml 2X Wash Buffer
- 15ml Elution Buffer

Storage Conditions: Store all DNA IQ™ System reagents at 22–25°C.



323TMB

Figure 2. Schematic of DNA isolation from stains on solid material using the DNA IQ™ System. See Section 3.B for a detailed protocol.




3238MB

Figure 3. Schematic of DNA purification from liquid samples using the DNA IQ™ System. See Section 3.C for a detailed protocol.

3. Protocol for the DNA IQ™ System

Materials to Be Supplied by the User

- 95–100% ethanol
- isopropyl alcohol
- 65°C heat block, water bath or thermal cycler
- 70°C heat block, water bath or thermal cycler (for stain or swab extraction)
- vortex mixer
- Microtubes, 1.5ml (Cat # V1231)
- DNA IQ™ Spin Baskets (Cat.# V1221)
- aerosol-resistant pipette tips
- MagneSphere® Technology Magnetic Separation Stand (twelve-position) (Cat.# Z5342)
- 1M DTT, for processing all samples except liquid blood
- Tissue and Hair Extraction Kit (for use with DNA IQ™) (Cat.# DC6740), for processing liquid blood samples
- nuclease-free water

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

3.A. Preparation of Reagents

The 1X Wash Buffer, Lysis Buffer and 1M DTT are required for DNA purification from all samples described in this Technical Bulletin. The stock Proteinase K solution and Incubation Buffer/Proteinase K solution are required to purify DNA from liquid blood (Section 3.C). The DTT, Proteinase K and Incubation Buffer are supplied with the Tissue and Hair Extraction Kit (for use with DNA IQ™).

Preparation of 1X Wash Buffer

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

For 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. Be sure bottle is closed tightly to prevent evaporation.

Preparation of Lysis Buffer

1. Determine the total volume of prepared Lysis Buffer to be used (Table 1), and add 1µl of 1M DTT for every 100µl of Lysis Buffer.

Table 1. Volume of Prepared Lysis Buffer Required Per Sample.

Sample	Lysis Buffer ¹	Lysis Buffer ²	Total Volume
Liquid blood (10–25µl)	195–225µl	100µl	295–325µl
1 Cotton swab	250µl	100µl	350µl
1/4th CEP swab	250µl	100µl	350µl
1–2 4mm punches of S&S 903 paper	150µl	100µl	250µl
1–3 2mm punches of FTA [®] paper	150µl	100µl	250µl
Cloth, up to 25mm ²	150µl	100µl	250µl

¹For use in Section 3.B, Step 2, or Section 3.C, Step 1.

²For use in Section 3.B, Step 9, or Section 3.C, Step 8.

- Mix by inverting several times.
- 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.

Note: If prepared Lysis Buffer forms a precipitate, warm solution to 37–60°C.

Preparation of Stock Proteinase K Solution

- 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.
- 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

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 freeze at –20°C.

Preparation of Incubation Buffer/Proteinase K Solution

- 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 80µl of Incubation Buffer/Proteinase K solution for each liquid blood sample (see Section 3.C).

Incubation Buffer	800µl
1M DTT	100µl
stock Proteinase K solution	100µl
total volume	1,000µl

- Mix gently, and store on ice during use.

Note: The final concentration of Proteinase K will be 1.8mg/ml.

3.B. DNA Isolation from Stains and Buccal Swabs

1. Place the appropriate amount of sample (Table 1) in a 1.5ml microcentrifuge tube.
2. Add the appropriate volume of prepared Lysis Buffer. Different samples require different volumes of Lysis Buffer; see Column 2 of Table 1 for the appropriate volume to add at this point. Close the lid, and incubate the tube at 70°C for 30 minutes.

For small stains, an alternative approach is to place the stained material in a DNA IQ™ Spin Basket (Cat.# V1221) seated in a 1.5ml Microtube (Cat.# V1231), and add 100-150µl of prepared Lysis Buffer to the basket. Carefully close the lid, and incubate at 70°C for 30 minutes. Most of the buffer should remain in the basket if the indicated tubes and spin baskets are used.

Proceed to Step 4.

Note: This alternative approach does not work reliably with samples requiring more than 150µl of prepared Lysis Buffer.

3. Remove the tube from the heat source, and transfer the prepared Lysis Buffer and sample to a DNA IQ™ Spin Basket seated in a 1.5ml microcentrifuge tube.
Note: It is important to centrifuge the prepared Lysis Buffer with the stained matrix to obtain maximum recovery.
4. Centrifuge at room temperature for 2 minutes at maximum speed in a microcentrifuge. Remove the spin basket.
5. Vortex the stock resin bottle for 10 seconds at high speed or until resin is thoroughly mixed. Add 7µl of DNA IQ™ Resin to the sample. Keep the stock resin resuspended while dispensing to obtain uniform results.
6. Vortex the sample/Lysis Buffer/resin mixture for 3 seconds at high speed. Incubate at room temperature for 5 minutes. Vortex mixture for 3 seconds once every minute during this 5-minute incubation.
7. Vortex tube for 2 seconds at high speed. Place tube in the magnetic 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.
8. Carefully remove and discard all of the solution without disturbing the resin pellet on the side of the tube.
Note: If some resin is drawn up in tip, gently expel resin back into tube to allow re-separation.
9. Add 100µl of prepared Lysis Buffer. Remove the tube from the magnetic stand, and vortex for 2 seconds at high speed.
10. Return tube to the magnetic stand, and discard all Lysis Buffer.

11. Add 100µl of prepared 1X Wash Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.
 12. Return tube to the magnetic stand, and discard all Wash Buffer.
 13. Repeat Steps 11 and 12 two more times for a total of three washes. Be sure that all of the solution has been removed after the last wash.
 14. 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.
 15. Add 100µl of Elution Buffer.
 16. Close the lid, and vortex the tube for 2 seconds at high speed. Incubate the tube at 65°C for 5 minutes.
 17. Remove the tube from the heat source, and vortex for 2 seconds at high speed. Immediately place the tube on the magnetic stand.
 - ⓘ Tubes must remain hot until placed in the magnetic stand or yield will decrease.
 18. Carefully transfer the DNA-containing solution to a container of choice.
- Note:** DNA can be stored at 4°C for short-term storage or at -20 or -70°C for long-term storage.

3.C. DNA Isolation from Liquid Blood

1. Vortex the stock resin bottle for 10 seconds at high speed or until resin is thoroughly mixed. For each sample, prepare the resin/Lysis Buffer mixture using 7µl of resin and the volume of prepared Lysis Buffer indicated below:

Volume of blood	Volume of DNA IQ™ Resin	Two volumes of prepared Lysis Buffer
10µl	7µl	195µl
15µl	7µl	205µl
20µl	7µl	215µl
25µl	7µl	225µl

2. Mix blood gently, and place 10–25µl in a 1.5ml microcentrifuge tube (15µl of blood can be used routinely).
3. Add 80µl of the Incubation Buffer/Proteinase K solution to the liquid blood sample. Incubate at 56°C for 1 hour.

3.C. DNA Isolation from Liquid Blood (continued)

- Vortex the resin/Lysis Buffer mixture for 2 seconds at high speed to ensure suspension of resin. Add the volume of resin/Lysis Buffer mixture indicated below to the sample. The resin/Lysis Buffer mixture should be mixed again if the resin begins to settle while dispensing aliquots.

Volume of blood	Volume of resin/Lysis Buffer mixture
10µl	202µl
15µl	212µl
20µl	222µl
25µl	232µl

- Vortex the sample/resin/Lysis Buffer mixture for 3 seconds at high speed. Incubate for 5 minutes at room temperature. Vortex mixture for 3 seconds once every minute during this 5-minute incubation.
- Vortex tube for 2 seconds at high speed. Place the tube in the magnetic 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.
- Carefully remove and discard all of the solution without disturbing the resin pellet on the side of the tube.
- Add 100µl of prepared Lysis Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.
- Return tube to the magnetic stand, and remove and discard all Lysis Buffer.
- Add 100µl of prepared 1X Wash Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.
- Return tube to the magnetic stand. Dispose of all Wash Buffer.
- Repeat Steps 10 and 11 two more times for a total of three washes. Be sure that all of the solution has been removed after the last wash.
- 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.

- Add 100µl of Elution Buffer.
- Close the lid, and vortex tube for 2 seconds at high speed. Incubate at 65°C for 5 minutes.
- Remove the tube from the heat source, and vortex for 2 seconds at high speed. Immediately place on the magnetic stand.

! Tubes must remain hot until placed in the magnetic stand, or yield will decrease.

17. Carefully transfer the DNA-containing solution to a container of choice.

Note: DNA can be stored at 4°C for short-term storage or at -20 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. E-mail: genetic@promega.com

<u>Symptoms</u>	<u>Causes and Comments</u>
Poor yield	Too much sample was used. Excessive amounts of sample can reduce the efficiency of DNA binding to the resin. Use less sample or more resin.
	Poor extraction. After heating stain in prepared Lysis Buffer, centrifuge buffer with matrix. Be sure enough liquid is present to wash out the DNA.
	Excessive drying of resin. Do not dry samples for more than 20 minutes, as overdrying the resin inhibits DNA elution.
Poor resin pellet formed	The resin settled before tube was placed in the magnetic stand. Samples should be placed in the magnetic stand immediately after mixing. Repeat mixing, and place tube in stand.
	Excessive input material was used relative to the recommended volumes of reagents. Use less initial sample. Consult protocols for recommended quantities of initial sample. Alternatively, use more resin per isolation. A proportional increase in resin will allow DNA capture from more initial sample. The increase in yield will be roughly proportional to the increase in resin.
Coloration in final wash or eluted solution (may affect results in downstream assays)	Insufficient washing. Remove all fluid during washes. Be sure that resin is completely resuspended during each wash step.
	Be sure a distinct resin pellet is formed during all washes.
	Use less initial sample.
	Perform additional washes with 1X Wash Buffer.

4. Troubleshooting (continued)

Symptoms	Causes and Comments
Resin present in final eluted solution (may affect results in downstream assays)	Resin is occasionally transferred by rapid pipetting or is caught in the meniscus of the final eluate. Vortex or mix solution, place in the magnetic stand and transfer eluate to new tube.
Low yield from FTA® blood card punches	DNA can be difficult to extract from FTA® blood card punches. If yields are low, increase the incubation temperature to 90°C in Section 3.B, Step 2.
Inconsistent yield (may affect results of downstream assays)	<p>Inconsistent amounts of resin. Vortex resin stock just before making aliquots. Be sure to vortex the resin/Lysis Buffer mixture while dispensing aliquots.</p> <p>Excessive drying of resin. Do not dry samples for more than 20 minutes, as overdrying the resin inhibits DNA elution.</p> <p>Too little sample. Use more initial sample. Consult protocols for recommended quantities of initial sample. If concentration of DNA is consistent, volume of eluted solution used in downstream assays can be modified.</p>

5. References

1. Greenspoon, S. and Ban, J. (2002) Robotic extraction of mock sexual assault samples using the Biomek® 2000 and the DNA IQ™ System. *Profiles in DNA* 5, 3-5.
2. Gill, P. *et al.* (1985) Forensic application of DNA 'fingerprints'. *Nature* 318, 577-9.

6. Composition of Buffers and Solutions

Elution Buffer

10mM Tris (pH 8.0)
0.1mM EDTA

7. Related Products

Product	Size	Cat.#
MagneSphere® Technology Magnetic Separation Stand (two-position)	1.5ml	Z5332
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	1.5ml	Z5342
PolyAtract® System 1000 Magnetic Separation Stand	1 each	Z5410
DNA IQ™ Spin Baskets*	1,000/pk	V1221
Microtubes, 1.5ml	1,000/pk	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
Slicprep™ 96 Device**	10 pack	V1391
AluQuant® Human DNA Quantitation System*	80 determinations	DC1010
	400 determinations	DC1011
Tissue and Hair Extraction Kit (for use with DNA IQ™)**	100 reactions	DC6740
DTT, Molecular Grade (Dry Powder)**	5g	V3151
	25g	V3155
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.

**For laboratory Use.

©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.

© 2001, 2002, 2006, 2009 Promega Corporation. All Rights Reserved.

AluQuant, MagneSphere, PolyATtract and PowerPlex are registered trademarks of Promega Corporation. DNA IQ is a trademark of Promega Corporation.

ART is a registered trademark of Molecular Bio-Products, Inc. Biomek is a registered trademark of Beckman Coulter, Inc. Freedom EVO is a registered trademark of Tecan AG Corporation. FTA is a registered trademark of Flinders Technologies, Pty, Ltd., and is licensed to Whatman. OliGreen and RiboGreen are registered trademarks of Molecular Probes, Inc. Quant-iT is a trademark of Molecular Probes, Inc.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.