



Promega

Technical Bulletin

DNA IQ™ System – Small Sample Casework Protocol

INSTRUCTIONS FOR USE OF PRODUCTS DC6700 AND DC6701.



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DNA IQ™ System – Small Sample Casework Protocol

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Technical Bulletin. Please contact Promega Technical Services if you have questions on use
of this system. E-mail: genetic@promega.com.

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1. Description

DNA analysis is playing an increasingly larger role in identifying both humans and animals. With the advent of large multiplexes such as the PowerPlex® 16 System, the amplification and analysis steps have been streamlined. However, the purification of DNA from a variety of samples is still a rate-limiting step in obtaining useful genotypes.

Several procedures are currently used to purify DNA from samples adsorbed to surfaces. Biological material must first be removed from these surfaces. This is typically done by soaking the material, which may result in inefficient recovery of small samples. The DNA must then be purified from inhibitors of PCR amplification and other components that may interfere with accurate quantitation methods.

Purification methods commonly used, such as phenol:chloroform extraction, use hazardous organic chemicals, require multiple centrifugations, may result in significant loss of material and can introduce amplification inhibitors. Chelex® extraction is rapid but frequently leaves amplification inhibitors. Purification

with silica matrices uses the affinity of DNA for silica and does not require organic components. Silica filters are convenient when used with a filtration system but tend to give lower yields and require extensive washing to remove the guanidine-based lysis buffer. Currently available silica magnetic particles tend to give higher yield but also need extensive washing.

The DNA IQ™ System^(a) uses a novel paramagnetic resin for DNA isolation. Using the DNA IQ™ System to process small casework samples requires two steps. For biological material on solid supports, the first step provides an easy, rapid, efficient and almost universal stain extraction method. This step is unnecessary for liquid samples. The second step uses the paramagnetic resin to purify DNA without requiring extensive washing to remove the lysis reagent. This system is designed to rapidly purify small quantities of DNA and give consistent yields for a specific sample type.

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:

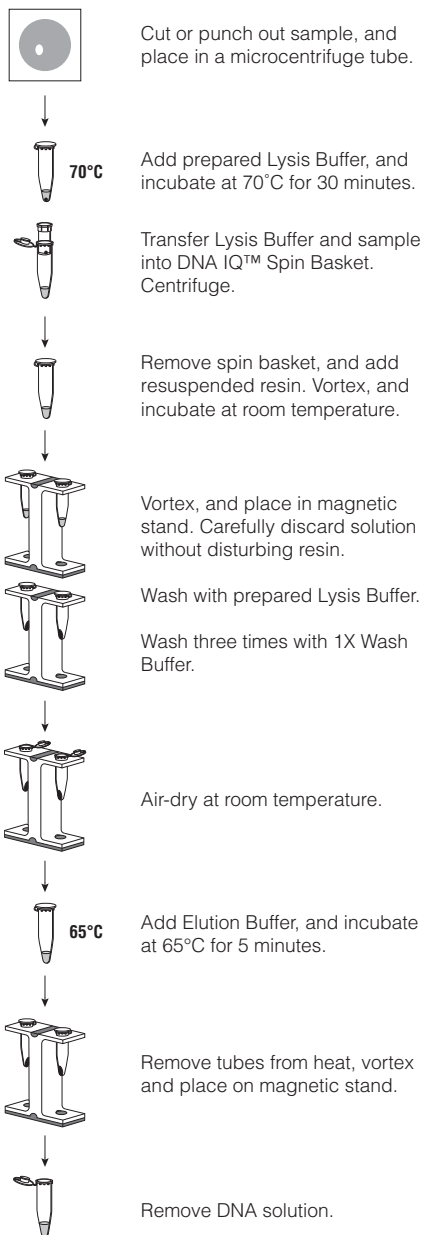
- 3ml Resin
- 150ml Lysis Buffer
- 70ml 2X Wash Buffer
- 50ml Elution Buffer

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

For Laboratory Use. This system includes:

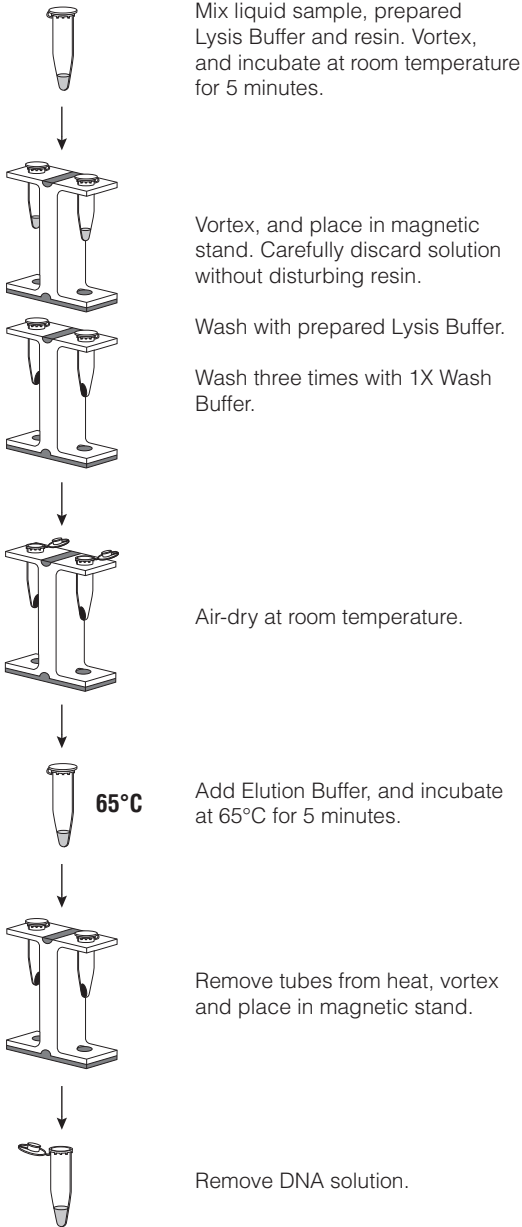
- 0.9ml Resin
- 40ml Lysis Buffer
- 30ml 2X Wash Buffer
- 15ml Elution Buffer

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



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Figure 1. Schematic of DNA isolation from stains on solid material using the DNA IQ™ System. See Section 4.B for a detailed protocol.



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Figure 2. Schematic of DNA purification from liquid samples using the DNA IQ™ System. See Section 4.C for a detailed protocol.

3. Sample Types Examined

DNA from the following sample types have been successfully purified at Promega or by external forensic laboratories. Due to the nature of casework samples (i.e, the samples may have been exposed to environmental factors for long periods of time and the amount of biological material may be limiting), DNA yields may vary, and DNA may not be obtained from all samples. Please see the most updated list at: www.promega.com/dnaiqsamples/. Tissue masses, including hair, bone, and sperm, require a proteinase K digestion to obtain reliable amounts of DNA. Contact Promega Technical Services (genetic@promega.com) for the latest information on available protocols.

Table 1. Types of Samples From Which DNA Has Been Successfully Isolated Using the DNA IQ™ System.

Sample Type	Promega	External	Comments
Fresh blood	Yes	Yes	Works with the following anti-clotting reagents: EDTA, citrate, heparin, ACD.
Frozen blood	Yes	Yes	Old blood may produce lower yields.
Bloodstains			
S&S 903 paper	Yes	Yes	
FTA® paper	Yes	Yes	
Cotton	Yes	Yes	
Blue denim	Yes	Yes	
Black denim	Yes	Yes	
Soil	Yes		
Leather	Yes	Yes	
Surface to swab		Yes	
Buccal swabs			
Cotton	Yes	Yes	
Rayon	Yes		
CEP paper	Yes		
Swab to FTA® paper		Yes	
Foam swab to paper		Yes	
Cigarette butt	Yes	Yes	Use paper wrapping; filter may form gel if heated with prepared Lysis Buffer.
Toothbrush		Yes	Soak bristles in prepared Lysis Buffer at 60°C for 30 minutes.
Envelope		Yes	Soak in 0.5% SDS before adding 2 volumes of prepared Lysis Buffer.
Urine		Yes	Sample from bladder cancer patient.

Table 2. Samples Requiring a Proteinase K Digestion Prior to Addition of Twice the Recommended Volume of Lysis Buffer.

Sample Type	Promega	External	Comments
Tissue			
Fresh	Yes	Yes	See the <i>Tissue and Hair Extraction Kit (for use with DNA IQ™) Technical Bulletin TB307.</i>
Formalin-fixed	Yes	Yes	See the <i>Tissue and Hair Extraction Kit (for use with DNA IQ™) Technical Bulletin TB307.</i>
Hair	Yes	Yes	See the <i>Tissue and Hair Extraction Kit (for use with DNA IQ™) Technical Bulletin TB307.</i>
Bone*		Yes	From pulverized bone samples.
Antler*		Yes	From drill shavings.
Differential extractions		Yes	See Section 4.D.

*Requires the Bone Incubation Buffer containing 1mg/ml proteinase K for DNA purification. Please contact Technical Services (genetic@promega.com) for a protocol for DNA isolation from bone samples.

4. Protocol for the DNA IQ™ System

Materials to Be Supplied by the User

- 95–100% ethanol
- isopropyl alcohol
- 1M DTT
- 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)
- proteinase K, to process samples listed in Table 2.

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

4.A. Preparation of Reagents

Preparation of 1X Wash Buffer

- 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.
- Replace cap, and mix by inverting several times.
- 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

- Determine the total volume of prepared Lysis Buffer to be used (Table 3), and add 1 μ l of 1M DTT for every 100 μ l of Lysis Buffer.

Note: Increasing the DTT concentration in the prepared Lysis Buffer to 60mM can improve the recovery of DNA from the sperm fraction of sperm-containing samples (Section 4.D). To prepare Lysis Buffer with a final concentration of 60mM DTT, add 6 μ l of 1M DTT to 100 μ l of Lysis Buffer.

Table 3. Volume of Prepared Lysis Buffer Required Per Sample.

Sample	Lysis Buffer ¹	Lysis Buffer ²	Total Volume
Liquid samples (up to 40 μ l)	100 μ l	100 μ l	200 μ l
1 Cotton swab	250 μ l	100 μ l	350 μ l
1/4th CEP swab	250 μ l	100 μ l	350 μ l
1–2 4mm punches of S&S 903 paper	150 μ l	100 μ l	250 μ l
1–3 2mm punches of FTA [®] paper	150 μ l	100 μ l	250 μ l
Cloth up to 25mm ²	150 μ l	100 μ l	250 μ l

¹For use in Section 4.B, Step 2, or Section 4.C, Step 1.

²For use in Section 4.B, Step 9, or Section 4.C, Step 7.

- 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.

4.B. DNA Isolation from Stains on Solid Material

The maximum DNA yield will depend on the sample type, even samples that contain DNA amounts in excess of the DNA-binding capacity of the resin. As expected for samples that do not contain DNA amounts exceeding the DNA-binding capacity, the yield will vary with the sample type and amount. Samples containing small amounts of DNA will have high efficiencies of recovery; as the DNA content approaches the maximum DNA -binding capacity, efficiency decreases (see Figure 1 of the *DNA IQ™ System – Database Protocol*, #TB297).

1. Place sample in a 1.5ml microcentrifuge tube (e.g. Microtubes, 1.5ml, Cat.# V1231).
2. Add the appropriate volume of prepared Lysis Buffer. Different samples require different volumes of prepared Lysis Buffer; see Column 2 of Table 3 for the appropriate volume to add at this point. Additional prepared Lysis Buffer may be used to cover entire sample. Close the lid, and incubate tube at 70°C for 30 minutes.

Exceptions:

- Heat-sensitive fabrics (e.g., polyester and nylon): Extract without heating.
- Leather: Lysis Buffer extraction with or without heat may not work on some leathers. Extract in a small volume of aqueous buffer (100–200µl), then add 2 volumes of Lysis Buffer after removing matrix.

Note: 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). 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.

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 Microtube.
4. Centrifuge at room temperature for 2 minutes at maximum speed in a microcentrifuge. Remove the spin basket.


Note: It is important to centrifuge the prepared Lysis Buffer and stained matrix to obtain maximum recovery.

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 resin resuspended while dispensing to obtain uniform results.
6. Vortex 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 100 μ l 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 100 μ l 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 25–100 μ l of Elution Buffer, depending on how much biological material was used. A lower elution volume ensures a higher final concentration of DNA.
 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.

4.C. DNA Isolation from Liquid Samples

This protocol is recommended for liquid samples, excluding liquid blood. A protocol for DNA purification from liquid blood can be found in the *DNA IQ™ System – Database Protocol #TB297*.

1. Prepare a stock solution of resin and Lysis Buffer by using the ratio of 7 μ l of resin to 93 μ l of prepared Lysis Buffer per sample (prepare extra to allow for losses during pipetting). The following equation will help determine the exact volumes to be made. Vortex the resin container for 10 seconds at high speed or until resin is thoroughly mixed.


$$(\text{Number of samples} + 1) \text{ ______ } \times 7\mu\text{l} = \text{______ } \mu\text{l of resin}$$

$$(\text{Number of samples} + 1) \text{ ______ } \times 93\mu\text{l} = \text{______ } \mu\text{l of prepared Lysis Buffer}$$

2. Mix liquid sample gently, and place an aliquot of up to 40 μ l into a 1.5ml microcentrifuge tube.
3. Vortex the resin/Lysis Buffer mixture for 3 seconds at high speed to ensure suspension of resin, and add 100 μ l of the mixture to the tube containing the liquid sample. The resin/Lysis Buffer mixture should be mixed again if the resin begins to settle while dispensing aliquots.
4. Vortex the sample/Lysis Buffer/resin mix for 3 seconds at high speed. Incubate 5 minutes at room temperature. Vortex mixture for 3 seconds once every minute during this 5-minute incubation.
5. Vortex 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 it 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 μ 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 remove and discard all Lysis Buffer.
9. Add 100 μ 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. Dispose of all Wash Buffer.
11. Repeat Steps 9 and 10 two more times for a total of three washes. Be 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 how much biological material was used. A 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.
-  Tubes must remain hot until placed in the magnetic stand, or yield will decrease.
16. 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.D. Processing of Sperm-Containing Samples

The following approach describes DNA purification from differentially extracted samples of sperm and nonsperm fractions using the DNA IQ™ System. This approach has been successfully performed in a forensic laboratory setting (1). The sample of interest is first processed using the laboratory's validated differential extraction protocol (2), including the extraction of biological material from the solid support, proteinase K digestion in the absence of DTT and pelleting and washing of the sperm cells. The following steps are to be followed once the sperm and nonsperm fractions have been separated.

There is no need to digest the sperm pellet with proteinase K and DTT, as the prepared Lysis Buffer in Step 1 will effectively disrupt the sperm cells after the initial proteinase K digestion.

Increasing the DTT concentration in the prepared Lysis Buffer to 60mM can improve the recovery of DNA from the sperm fraction. To prepare Lysis Buffer with a final concentration of 60mM DTT, add 6µl of 1M DTT to 100µl of DNA IQ™ Lysis Buffer instead of 1µl of 1M DTT as described in Section 4.A.

1. Add at least 2 volumes (minimum 100µl) of prepared Lysis Buffer and 7µl of resin to the sperm pellet.
2. Vortex for 3 seconds at high speed, and incubate at room temperature for 5 minutes.
3. Proceed to Steps 5–16 of Section 4.C to purify DNA from this sperm fraction.
4. Add two volumes of prepared Lysis Buffer and 7µl of resin to the nonsperm fraction.

Note: If desired, 100µl of a 500µl extraction can be processed. This amount typically gives sufficient DNA for analysis.

5. Vortex for 3 seconds at high speed, and incubate at room temperature for 5 minutes.
6. Proceed to Steps 5-16 of Section 4.C to purify DNA from this nonsperm fraction.

5. 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	<p>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.</p> <p>Poor extraction. After heating stain in prepared Lysis Buffer, centrifuge buffer with matrix. Be sure enough liquid is present to wash out the DNA.</p> <p>Excessive drying of resin. Do not dry samples for more than 20 minutes, as overdrying the resin inhibits DNA elution.</p> <p>For sperm-containing samples, increasing the DTT concentration in the prepared Lysis Buffer to 60mM may improve yield (see Section 4.D).</p>
Poor resin "pellet" formed	<p>The resin settled before the 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.</p> <p>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.</p>
Coloration in final wash or eluted solution (may affect results of downstream assays)	<p>Insufficient washing. Remove all fluid during washes. Be sure that resin is completely resuspended during each wash step.</p> <p>Be sure a distinct resin pellet is formed during all washes.</p> <p>Use less initial sample.</p> <p>Perform additional washes with 1X Wash Buffer.</p>

5. Troubleshooting (continued)

Symptoms	Causes and Comments
Resin present in final eluted solution (may affect results of 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.
Inconsistent yield (may affect results from downstream assays)	Inconsistent amounts of resin. Vortex resin stock just before making aliquots. Be sure to vortex the resin/Lysis Buffer mixture while dispensing aliquots.
	Excessive drying of resin. Do not dry samples for more than 20 minutes, as overdrying the resin inhibits DNA elution.
	Contaminating nonhuman DNA in the initial sample can decrease yield of human DNA. The DNA IQ™ System captures total DNA, including single- and double-stranded DNA.

6. References

- 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.
- Gill, P. *et al.* (1985) Forensic application of DNA 'fingerprints'. *Nature* 318, 577-9.

7. Composition of Buffers and Solutions

Elution Buffer

10mM Tris (pH 8.0)
0.1mM EDTA

Bone Incubation Buffer

10mM Tris (pH 8.0)
100mM NaCl
50mM EDTA
0.5% SDS

8. 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
DTI, 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.

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