

April 2010

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# Generation™ Capture Column Handbook

For purification of DNA from

whole blood

bone marrow

buffy coat

body fluids

cultured cells

cell suspensions

Gram-negative bacteria



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Sample & Assay Technologies

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## Kit Contents

<b>Generation Capture Column Kit</b>	<b>(50)</b>	<b>(300)</b>
<b>Catalog no.</b>	<b>159914</b>	<b>159916</b>
<b>Number of preps</b>	<b>50</b>	<b>300</b>
DNA Purification Solution 1	44 ml	250 ml
DNA Elution Solution 2	22 ml	125 ml
Waste Collection Tubes	100	600
DNA Collection Tubes	50	300
Generation Capture Columns	50	300
Handbook	1	1

## Storage

The components of the Generation Capture Column Kit should be stored dry at room temperature (15–25°C). All kit components can be stored under these conditions until the expiration date on the kit box without showing any reduction in performance.

## Product Use Limitations

The Generation Capture Column Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

## Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish.

Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit [www.qiagen.com](http://www.qiagen.com) ).

## **Technical Assistance**

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the Generation Capture Column Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major 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 QIAGEN. 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 call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://www.qiagen.com) ).

## **Quality Control**

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Generation Capture Column Kits is tested against predetermined specifications to ensure consistent product quality.

## **Safety Information**

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at [www.qiagen.com/ts/msds.asp](http://www.qiagen.com/ts/msds.asp) where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

### **24-hour emergency information**

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel.: +49-6131-19240

## Introduction

The Generation Capture Column Kit provides the components and procedures necessary for purifying genomic DNA from whole blood, bone marrow, buffy coat, body fluids, cultured cells, cell suspensions, and Gram-negative bacteria.

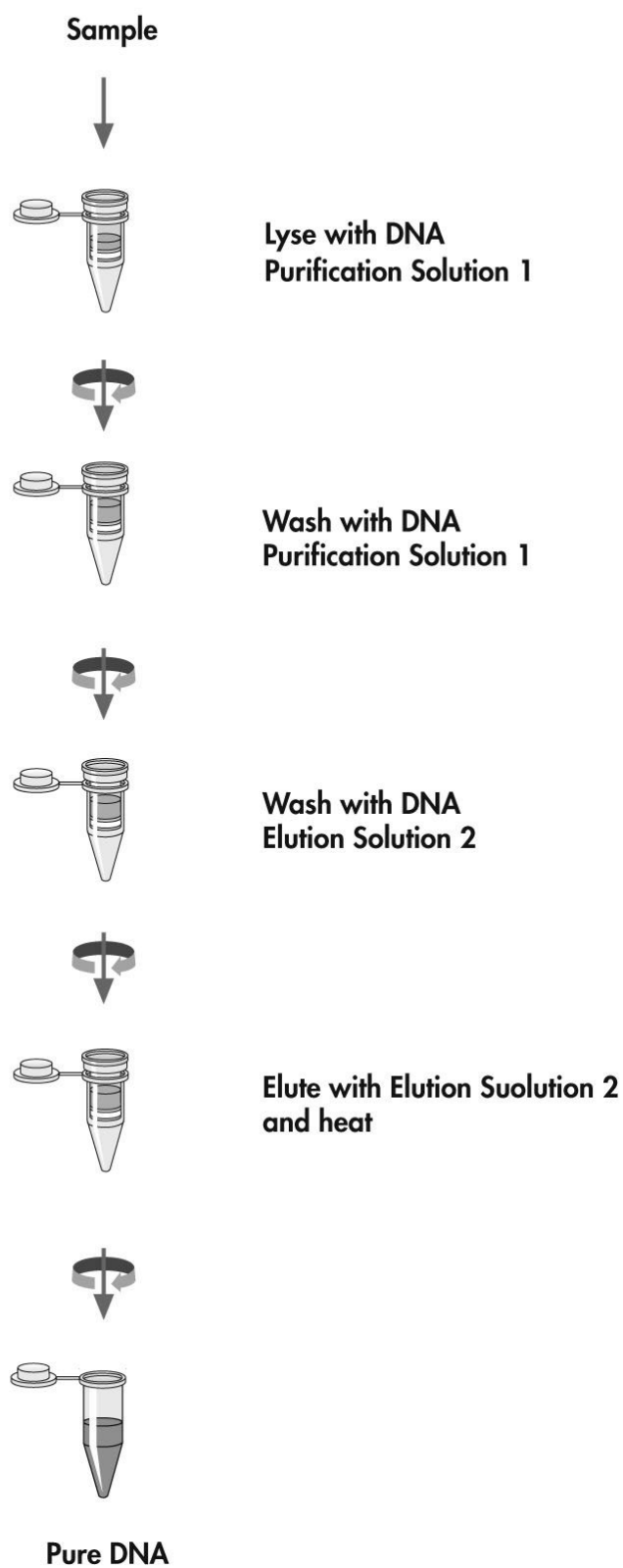
The Generation Capture Column Kit is used to purify DNA primarily for subsequent amplification by polymerase chain reaction (PCR) or other DNA amplification technology. The rapid and easy-to-use method produces consistently high DNA quality and yield for these applications. The Generation Capture Column Kit employs a flexible solid-phase system with a unique trifunctional purification matrix and reagents for lysing cells and capturing and releasing DNA. This method does not require prelysing of the sample or precipitation of the DNA.

The Generation Capture Column Kit is based on an optimized system that uses two reagents, DNA Purification Solution 1 and DNA Elution Solution 2, and a specially formulated purification matrix.

## Principle and procedure

A sample is applied to the purification matrix contained in a spin column. The cells contained in the sample lyse upon contact with the matrix. Once the cells are lysed, DNA is captured by the matrix material which makes it possible to efficiently wash away contaminants, leaving the DNA bound to the matrix. Contaminants, including protein, heme, and RNA are removed from the matrix by washing with DNA Purification Solution 1. Following removal of contaminants, the DNA is released from the matrix with DNA Elution Solution 2 and heat. Samples of purified DNA are ready for analysis and do not require precipitation. The purified DNA solution is compatible with PCR and other DNA amplification technology but is not compatible with real-time PCR, restriction enzyme digests or Southern blot analysis.

## Generation Capture Column Procedure





## Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Microcentrifuge
- Incubator or dry block heater
- Pipets with tips

### For processing frozen samples

- Water bath at 37°C
- Ice

### For processing cell suspensions from tissue homogenates

- Ice
- 1.5 ml microcentrifuge tube
- Microcentrifuge tube pestle
- PBS with EDTA (8 g NaCl, 0.2 g KCl, 2.72 g  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 0.24 g  $\text{KH}_2\text{PO}_4$ , 0.372 g EDTA disodium salt, dissolved in ultrapure water, brought up to a volume of 1000 ml and autoclaved)

## Important Notes

### Starting material

Each Generation Capture Column well can bind up to 60  $\mu\text{g}$  nucleic acids, but yield depends on sample volume and nucleic acid content. The procedure is optimized for use with a starting volume of 200  $\mu\text{l}$  containing up to  $1 \times 10^7$  cells.

### Storage of blood samples

Fresh or frozen whole blood samples treated with EDTA or citrate can be used. Yield and quality of the purified DNA depend on the storage conditions of the blood. Fresher blood samples yield better results.

For short-term storage (up to 10 days), collect blood in tubes containing EDTA as an anticoagulant, and store the tubes at 2–8°C. For long-term storage, collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required). Store the blood frozen at –70°C.

### Yield and quality of purified DNA

DNA yields depend on the number of nucleated cells present. Yields from whole blood may vary widely since white blood cell counts can differ by as much as tenfold. Typical DNA yields obtained from 200  $\mu\text{l}$  whole blood with a normal white blood cell count ( $5\text{--}7 \times 10^6$  cells/ml blood) are in the range of 3–8  $\mu\text{g}$  DNA.

The Generation Capture Column procedure yields pure DNA, indicated by  $A_{260}/A_{280}$  ratios greater than 1.4. Up to 95% of the purified DNA is greater than 23 kb in size. The purified DNA can be stored at 2–8°C for up to 3 months. For long term storage (>3 months) store DNA at –20°C.

# Protocol: DNA Purification from Whole Blood and Bone Marrow

## Things to do before starting

- Preheat block or bath to 99°C.
- Frozen blood and bone marrow samples should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.

## Procedure

- 1. For each sample to be processed assemble the following: 1 Capture Column contained in a blue Waste Collection Tube, 1 additional blue Waste Collection Tube and 1 clear DNA Collection Tube. Label tubes.**
- 2. Add 200  $\mu$ l well-mixed sample to the Capture Column by gently touching the center of the matrix with the pipet tip during dispensing.**
- 3. Incubate at room temperature to allow the DNA to adsorb.**  
Incubate for at least 1 min or up to 1 h. The sample may not completely saturate the column.
- 4. Add 400  $\mu$ l DNA Purification Solution 1.**  
Incubate 1 min at room temperature.  
The solution may begin draining into blue Waste Collection Tube during the incubation.
- 5. Centrifuge 10 s at 2000–12,000  $\times$  g.**  
A waste volume of 600  $\mu$ l will be collected in the blue Waste Collection Tube.
- 6. Transfer the Capture Column to the second blue Waste Collection Tube.**
- 7. Add an additional 400  $\mu$ l DNA Purification Solution 1.**
- 8. Incubate 1 min at room temperature.**
- 9. Centrifuge 10 s at 2000–12,000  $\times$  g.**  
A waste of volume of 400  $\mu$ l will be collected. The matrix should be white or nearly white.
- 10. Add 200  $\mu$ l DNA Elution Solution 2; no incubation is required.**
- 11. Centrifuge 10 s at 2000–12,000  $\times$  g to collect an additional 200  $\mu$ l waste.**  
The matrix containing the purified DNA should be white.

**12. Transfer the Capture Column to clear DNA Collection Tube and discard the second blue Waste Collection Tube containing 600  $\mu$ l waste solution.**

**13. Add 100  $\mu$ l DNA Elution Solution 2.**

**14. Incubate at 99°C for 10 min.**

Position tube so that the Capture Column is completely contained within the heat block to ensure proper heating.

**15. Centrifuge 20 s at 2000–12,000 x g immediately following heating step.**

Purified DNA will be released from the Capture Column. The 100  $\mu$ l DNA solution should appear clear.

The purified DNA is ready for analysis.

# Protocol: DNA Purification from Buffy Coat

## Things to do before starting

- Preheat block or bath to 99°C.
- Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy coat fraction from whole blood is simple and yields approximately 5–10 times more DNA than an equivalent volume of whole blood. Prepare buffy coat by centrifuging whole blood at 2500 x g for 10 min at room temperature. After centrifugation, three different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. DNA may be purified from up to 200  $\mu$ l buffy coat preparation containing a maximum of  $1 \times 10^7$  white blood cells.
- Frozen buffy coat should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.

## Procedure

- 1. For each sample to be processed assemble the following: 1 Capture Column contained in a blue Waste Collection Tube, 1 additional blue Waste Collection Tube and 1 clear DNA Collection Tube. Label tubes.**
- 2. Add 200  $\mu$ l well-mixed sample to the Capture Column by gently touching the center of the matrix with the pipet tip during dispensing.**
- 3. Incubate at room temperature to allow the DNA to adsorb.**  
Incubate for at least 1 min or up to 1 h. The sample may not completely saturate the column.
- 4. Add 400  $\mu$ l DNA Purification Solution 1.**  
Incubate 1 min at room temperature.  
The solution may begin draining into blue Waste Collection Tube during the incubation.
- 5. Centrifuge 10 s at 2000–12,000 x g.**  
A waste volume of 600  $\mu$ l will be collected in the blue Waste Collection Tube.
- 6. Transfer the Capture Column to the second blue Waste Collection Tube.**
- 7. Add an additional 400  $\mu$ l DNA Purification Solution 1.**
- 8. Incubate 1 min at room temperature.**

**9. Centrifuge 10 s at 2000–12,000 x g.**

A waste volume of 400  $\mu$ l will be collected. The matrix should be white or nearly white.

**10. Add 200  $\mu$ l DNA Elution Solution 2; no incubation is required.**

**11. Centrifuge 10 s at 2000–12,000 x g to collect an additional 200  $\mu$ l waste.**

The matrix containing the purified DNA should be white.

**12. Transfer the Capture Column to clear DNA Collection Tube and discard the second blue Waste Collection Tube containing 600  $\mu$ l waste solution.**

**13. Add 100  $\mu$ l DNA Elution Solution 2.**

**14. Incubate at 99°C for 10 min.**

Position tube so that the Capture Column is completely contained within the heat block to ensure proper heating.

**15. Centrifuge 20 s at 2000–12,000 x g immediately following heating step.**

Purified DNA will be released from the Capture Column. The 100  $\mu$ l DNA solution should appear clear.

The purified DNA is ready for analysis.

# Protocol: DNA Purification from Body Fluids

## Things to do before starting

- Preheat block or bath to 99°C.
- Body fluids with low cell numbers might require concentration by centrifuging the sample. Pellet cells from 3–40 ml body fluid by centrifuging at 2000 x g for 10 min. Remove the supernatant, leaving 200  $\mu$ l residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down 10 times. Place the sample on ice for immediate use or store frozen at –80°C.
- Frozen body fluid samples should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.

## Procedure

- 1. For each sample to be processed assemble the following: 1 Capture Column contained in a blue Waste Collection Tube, 1 additional blue Waste Collection Tube and 1 clear DNA Collection Tube. Label tubes.**
- 2. Add 200  $\mu$ l well-mixed sample to the Capture Column by gently touching the center of the matrix with the pipet tip during dispensing.**
- 3. Incubate at room temperature to allow the DNA to adsorb.**  
Incubate for at least 1 min or up to 1 h. The sample may not completely saturate the column.
- 4. Add 400  $\mu$ l DNA Purification Solution 1.**  
Incubate 1 min at room temperature.  
The solution may begin draining into blue Waste Collection Tube during the incubation.
- 5. Centrifuge 10 s at 2000–12,000 x g.**  
A waste volume of 600  $\mu$ l will be collected in the blue Waste Collection Tube.
- 6. Transfer the Capture Column to the second blue Waste Collection Tube.**
- 7. Add an additional 400  $\mu$ l DNA Purification Solution 1.**
- 8. Incubate 1 min at room temperature.**
- 9. Centrifuge 10 s at 2000–12,000 x g.**  
A waste of volume of 400  $\mu$ l will be collected. The matrix should be white or nearly white.
- 10. Add 200  $\mu$ l DNA Elution Solution 2; no incubation is required.**

**11. Centrifuge 10 s at 2000–12,000 x g to collect an additional 200  $\mu$ l waste.**

The matrix containing the purified DNA should be white.

**12. Transfer the Capture Column to clear DNA Collection Tube and discard the second blue Waste Collection Tube containing 600  $\mu$ l waste solution.**

**13. Add 100  $\mu$ l DNA Elution Solution 2.**

**14. Incubate at 99°C for 10 min.**

Position tube so that the Capture Column is completely contained within the heat block to ensure proper heating.

**15. Centrifuge 20 s at 2000–12,000 x g immediately following heating step.**

Purified DNA will be released from the Capture Column. The 100  $\mu$ l DNA solution should appear clear.

The purified DNA is ready for analysis.



# Protocol: DNA Purification from Cultured Cells and Cell Suspensions

## Things to do before starting

- Preheat block or bath to 99°C.
- Cultured cells can be used fresh or frozen. Collect suspended cultured cells and place on ice until use. Determine the number of cells using a hemacytometer or other cell counter. A 200  $\mu\text{l}$  suspension containing up to  $1 \times 10^7$  cultured cells may be added directly to the Capture Plate.
- Cell cultures with low cell numbers might require concentration by centrifuging the sample. Pellet cells by centrifuging at 13,000–16,000  $\times g$  for 5 s. Remove the supernatant, leaving 200  $\mu\text{l}$  residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down 10 times. Place the sample on ice for immediate use or store frozen at  $-80^\circ\text{C}$ .
- Cell suspensions prepared from tissue homogenates can be used either fresh or frozen. Collect fresh samples quickly and keep on ice at all times to reduce DNase activity. Add 20 mg tissue to a 1.5 ml microcentrifuge tube containing 300  $\mu\text{l}$  cold PBS (preferably containing 1 mM EDTA to reduce DNase activity). Homogenize quickly using 20 strokes with a microcentrifuge tube pestle. Place sample on ice and allow cell clumps to settle for 2–10 min. Alternatively, the sample may be centrifuged for 1–3 s at the lowest speed  $<2000 \times g$ . Carefully remove the upper 200  $\mu\text{l}$  cell suspension excluding any cell clumps.
- Frozen cells should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.

## Procedure

1. **Harvest cells according to steps 1a (for cells grown in suspension) or 1b (for cells grown in a monolayer).**
  - 1a. **Cells grown in suspension (do not use more than  $1 \times 10^7$  cells): Determine the number of cells. Centrifuge the appropriate number of cells for 5 min at 300  $\times g$  in a 1.5 ml microcentrifuge tube. Remove the supernatant leaving behind 200  $\mu\text{l}$  residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down until the cells are resuspended. Continue with step 2.**

- 1b. Cells grown in a monolayer (do not use more than  $1 \times 10^7$  cells):**  
Cells grown in a monolayer can be detached from the culture flask by either trypsinization or using a cell scraper.

**To trypsinize cells:**

**Aspirate the medium and wash cells with PBS.\* Aspirate the PBS and add 0.10–0.25% trypsin.\* After cells have detached from the dish or flask, collect them in medium,\* and transfer the appropriate number of cells (maximum  $1 \times 10^7$  cells) to a 1.5 ml microcentrifuge tube. Centrifuge for 5 min at 300 x g. Remove the supernatant leaving behind 200  $\mu$ l residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down until the cells are resuspended. Continue with step 2.**

**Using a cell scraper:**

**Detach cells from the dish or flask. Transfer the appropriate number of cells (maximum  $1 \times 10^7$  cells) to a 1.5 ml microcentrifuge tube and centrifuge for 5 min at 300 x g. Remove the supernatant leaving behind 200  $\mu$ l residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down until the cells are resuspended. Continue with step 2.**

- 2. For each sample to be processed assemble the following: 1 Capture Column contained in a blue Waste Collection Tube, 1 additional blue Waste Collection Tube and 1 clear DNA Collection Tube. Label tubes.**
- 3. Add 200  $\mu$ l well-mixed sample to the Capture Column by gently touching the center of the matrix with the pipet tip during dispensing.**
- 4. Incubate at room temperature to allow the DNA to adsorb.**  
Incubate for at least 1 min or up to 1 h. The sample may not completely saturate the column.
- 5. Add 400  $\mu$ l DNA Purification Solution 1.**  
Incubate 1 min at room temperature.  
The solution may begin draining into blue Waste Collection Tube during the incubation.
- 6. Centrifuge 10 s at 2000–12,000 x g.**  
A waste volume of 600  $\mu$ l will be collected in the blue Waste Collection Tube.
- 7. Transfer the Capture Column to the second blue Waste Collection Tube.**
- 8. Add an additional 400  $\mu$ l DNA Purification Solution 1.**
- 9. Incubate 1 min at room temperature.**

\*When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

**10. Centrifuge 10 s at 2000–12,000 x g.**

A waste of volume of 400  $\mu$ l will be collected. The matrix should be white or nearly white.

**11. Add 200  $\mu$ l DNA Elution Solution 2; no incubation is required.**

**12. Centrifuge 10 s at 2000–12,000 x g to collect an additional 200  $\mu$ l waste.**

The matrix containing the purified DNA should be white.

**13. Transfer the Capture Column to clear DNA Collection Tube and discard the second blue Waste Collection Tube containing 600  $\mu$ l waste solution.**

**14. Add 100  $\mu$ l DNA Elution Solution 2.**

**15. Incubate at 99°C for 10 min.**

Position tube so that the Capture Column is completely contained within the heat block to ensure proper heating.

**16. Centrifuge 20 s at 2000–12,000 x g immediately following heating step.**

Purified DNA will be released from the Capture Column. The 100  $\mu$ l DNA solution should appear clear.

The purified DNA is ready for analysis.

# Protocol: DNA Purification from Gram-Negative Bacteria

## Things to do before starting

- Preheat block or bath to 99°C.
- Gram-negative bacterial cultures can be used either fresh or frozen. Typically, an overnight culture contains  $1\text{--}3 \times 10^9$  cells per ml. Due to the small genome size of Gram-negative bacteria, up to  $3 \times 10^9$  cells may be applied to the column for DNA purification. Thus, culture can either be used directly, or, if necessary, concentrated by centrifuging. To concentrate, pellet 1 ml of overnight culture at 13,000–16,000  $\times g$  for 1 min. Remove the supernatant, leaving 200  $\mu\text{l}$  residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down 10 times. Place the sample on ice for immediate use or store frozen at  $-80^\circ\text{C}$ .
- Frozen bacterial samples should be thawed and equilibrated to room temperature (15–25°C) before beginning the procedure.

## Procedure

1. **For each sample to be processed assemble the following: 1 Capture Column contained in a blue Waste Collection Tube, 1 additional blue Waste Collection Tube and 1 clear DNA Collection Tube. Label tubes.**
2. **Add 200  $\mu\text{l}$  well-mixed sample to the Capture Column by gently touching the center of the matrix with the pipet tip during dispensing.**
3. **Incubate at room temperature to allow the DNA to adsorb.**  
Incubate for at least 1 min or up to 1 h. The sample may not completely saturate the column.
4. **Add 400  $\mu\text{l}$  DNA Purification Solution 1.**  
Incubate 1 min at room temperature.  
The solution may begin draining into blue Waste Collection Tube during the incubation.
5. **Centrifuge 10 s at 2000–12,000  $\times g$ .**  
A waste volume of 600  $\mu\text{l}$  will be collected in the blue Waste Collection Tube.
6. **Transfer the Capture Column to the second blue Waste Collection Tube.**
7. **Add an additional 400  $\mu\text{l}$  DNA Purification Solution 1.**
8. **Incubate 1 min at room temperature.**

**9. Centrifuge 10 s at 2000–12,000 x g.**

A waste of volume of 400  $\mu$ l will be collected. The matrix should be white or nearly white.

**10. Add 200  $\mu$ l DNA Elution Solution 2; no incubation is required.**

**11. Centrifuge 10 s at 2000–12,000 x g to collect an additional 200  $\mu$ l waste.**

The matrix containing the purified DNA should be white.

**12. Transfer the Capture Column to clear DNA Collection Tube and discard the second blue Waste Collection Tube containing 600  $\mu$ l waste solution.**

**13. Add 100  $\mu$ l DNA Elution Solution 2.**

**14. Incubate at 99°C for 10 min.**

Position tube so that the Capture Column is completely contained within the heat block to ensure proper heating.

**15. Centrifuge 20 s at 2000–12,000 x g immediately following heating step.**

Purified DNA will be released from the Capture Column. The 100  $\mu$ l DNA solution should appear clear.

The purified DNA is ready for analysis.

## Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or molecular biology applications (for contact information, see back cover or visit [www.qiagen.com](http://www.qiagen.com) ).

### Comments and suggestions

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#### Capture Column is not white after elution.

- |  |  |
|--|--|
| a) Concentrated buffy coat, bone marrow or tissue samples were used                                    | Slight off-white coloration may be seen in the column with concentrated buffy coat, bone marrow or tissue samples. This should not affect subsequent analyses.   |
| b) Capture Column was stored improperly or sample was not mixed well before applying to Capture Column | If necessary, repeat purification as follows: <ul style="list-style-type: none"><li>■ Discard the 600 <math>\mu</math>l waste solution from the second blue Waste Collection Tube. Add another 400 <math>\mu</math>l DNA Purification Solution 1 to the Capture Column and incubate 1 min at room temperature.</li><li>■ Centrifuge 10 s at 2000–12,000 x g.</li><li>■ Add 200 <math>\mu</math>l DNA Elution Solution 2 to the Capture Column; no incubation is required.</li><li>■ Centrifuge 10 s at 2000–12,000 x g.</li><li>■ Elute DNA from Capture Column by following steps 13–14 of the protocol on page 12.</li></ul> |

#### Low DNA yield

- |  |   |
|--|---|
| a) Collection Tube does not fit securely into the heat block | If the Capture Column is not completely contained within the heat block it will not be heated to the correct temperature which will result in a reduced yield of DNA. |
|--|---|

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## Comments and suggestions

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- b) Purification matrix was allowed to cool before centrifugation to elute the DNA
- c) Not enough cells in the starting material
- d) DNA Purification Solution 1 was added instead of DNA Elution Solution 2 during the elution step
- Samples should be centrifuged while warm to obtain the maximum DNA yield. Allowing a Capture Column to cool for more than 5 min will reduce DNA yields by approximately 25%.
- If possible, count the cells prior to adding sample to the purification matrix to assure that a sufficient number of cells are used. If necessary, increase the number of cells in the sample by centrifuging to concentrate the sample prior to beginning the DNA purification procedure.
- DNA Purification Solution 1 will not release DNA from the purification matrix. Recover DNA as follows:
- Centrifuge sample at 2000–12,000 x g to collect DNA Purification Solution 1 in the Sample Collection Tube and discard the flow through.
  - Elute DNA by following steps 13–14 of the protocol on page 12.

### Little or no DNA in eluate

Large elution volume was used

Elute DNA sample with 100  $\mu$ l rather than 200  $\mu$ l DNA Elution Solution 1. Eluting the DNA using 100  $\mu$ l DNA Elution Solution 1 should result in the same yield but the concentration will be increased approximately twofold.

### Low $A_{260}/A_{280}$ ratio for purified DNA

- a) The purification matrix was overloaded

Capture Columns are designed to purify DNA from up to  $1 \times 10^7$  cells in a 200  $\mu$ l sample volume. Adding more than  $1 \times 10^7$  cells or sample volumes greater than 200  $\mu$ l may overload the purification chemistry inhibiting complete cell lysis and resulting in a low yield and low  $A_{260}/A_{280}$ .

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**Comments and suggestions**

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b) DNA purified from a sample that was stored improperly

This should not affect amplification results.



## **Appendix: Determination of Concentration, Yield, and Purity**

### **Determination of concentration, yield, and purity**

DNA yields are determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. DNA purified with the Generation Capture Plate procedure should have a ratio of 1.4–1.9. The purified DNA solution is compatible with PCR and other DNA amplification technologies but is not compatible with real-time PCR, restriction enzyme digests or Southern blot analysis.

Absorbance readings at 260 nm should lie between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220–320 nm (a scan will show if there are other factors affecting absorbance at 260 nm). Both DNA and RNA are measured with a spectrophotometer. To measure only DNA, a fluorometer must be used.

### **References**

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

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Product	Contents	Cat. no.
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<b>Related products</b>		
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Generation Capture Plate Kit (12)	For 12 x 96 preps: 12 Capture Plate Sets, 36 Plate Covers, 24 Cap Mats, and reagents	159932

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