Second Edition April 2010

Generation™ Capture Plate Handbook

For high-throughput purification of DNA from

- whole blood
- bone marrow
- buffy coat
- body fluids
- cultured cells
- cell suspensions
- Gram-negative bacteria

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
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- microRNA research and RNAi
- Automation of sample and assay technologies

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Generation Capture Plate Kit	**(12)**
Catalog no.	**159932**
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Plate Covers	36
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DNA Purification Solution 1	1000 ml
DNA Elution Solution 2	500 ml
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Storage

The components of the Generation Capture Plate 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 Plate 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).

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 sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the Generation Capture Plate 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 see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Generation Capture Plate Kit 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/support/MSDS.aspx 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 Plate Kit provides the components and procedures necessary for purifying genomic DNA from whole blood, bone marrow, buffy coat, body fluids, cultured cells, cells in suspension, and Gram-negative bacteria.

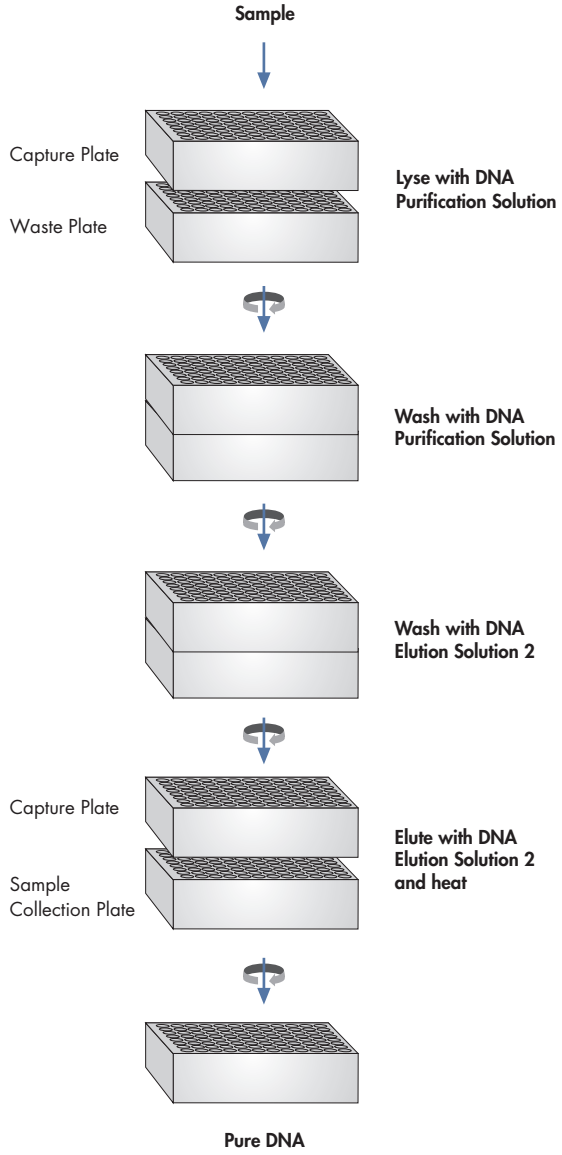
The Generation Capture Plate Kit is used to purify DNA primarily for subsequent amplification by polymerase chain reaction (PCR) or other DNA amplification technologies. The rapid and easy-to-use method produces consistently high DNA quality and yield for these applications. The Generation Capture Plate 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 Plate 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 96-well plate. 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 technologies but is not compatible with real-time PCR, restriction enzyme digests, or Southern blot analysis.

Generation Capture Plate 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.

- Centrifuge 4-15C or 4K15C*
- Plate Rotor 2 x 96
- Microwave oven (see recommendations in the protocol) and protective equipment, such as hot hands or mitts
- Multichannel pipets with tips

  For efficient processing, we recommend the use of an electric multichannel pipet with a minimum capacity of 650 µl per pipet tip, such as Matrix® Impact® cordless electronic multichannel pipets. These have a unique expandable tip-spacing system, allowing the user to transfer liquid directly from test tubes to microtiter plates. Extended tips (Matrix, cat. no. 8255) are suitable for use with these pipets. For ordering information, please contact Matrix Technologies Corporation, USA, or its subsidiaries or distributors in other countries.
- Reagent reservoirs for multichannel pipet
- When processing one plate at a time: Water to balance plates

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 $Na_2HPO_4 \cdot 7H_20$, 0.24 g KH_2PO_4, 0.372 g EDTA disodium salt, dissolved in ultrapure water, brought up to a volume of 1000 ml and autoclaved)

* The Centrifuge 4-15 from QIAGEN and freely programmable models of centrifuges 4-15, 4K15, 6-10, 6K10, 6-15, and 6K15 from Sigma Laborzentrifugen GmbH can also be used.

Important Notes

Starting material

Each Generation Capture Plate well can bind up to 60 µg nucleic acids, but yield depends on sample volume and nucleic acid content. The procedure is optimized for use with a starting volume of 200 µl containing up to 1 x 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 µl whole blood with a normal white blood cell count (5–7 x 10^6 cells/ml blood) are in the range of 3–8 µg DNA.

The Generation Capture Plate 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.

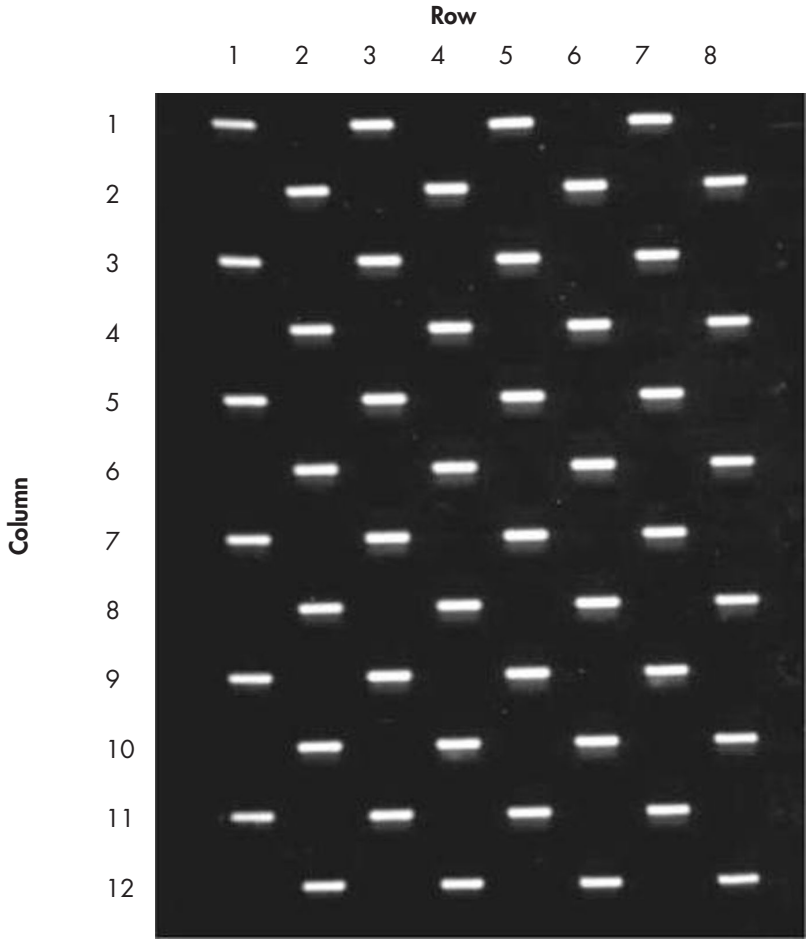


Figure 1. Cross-contamination-free PCR amplification. Whole blood or PBS (200 µl) was dispensed into alternate wells of a Generation Capture Plate. DNA was purified using the Generation Capture Plate Kit and PCR amplified using HLA-H locus primers, which are routinely used to detect the presence of DNA-quantity equivalents of one cell. A 10 µl volume of the reaction with ethidium bromide staining (125 µg/ml) was subjected to electrophoresis in a 96-well gel system at 80 V for 5 minutes. The 390 bp HLA-H amplicon was detected in all 48 positive controls. No cross-contamination was detected in any of the 48 negative controls.

Centrifugation

Centrifugation of kit plates is performed at 2000 x *g*.

Note: When using the refrigerated Centrifuge 4K15C, set the temperature to 40°C for all centrifugation steps.

Abbreviated instructions for using the Centrifuge 4-15C

1. **Switch on the centrifuge by pressing the main switch on the back.**
2. **Select the rotor selection list in the display field by turning the knob. Press the knob and turn it again to select the rotor/bucket combination "09100/09158" for the Plate Rotor 2 x 96. Confirm entry by pressing the knob.**

   Entering the rotor number automatically sets the time and speed limits for centrifugation for that rotor, eliminating the danger of the centrifuge running too fast.
3. **Select "RCF" by turning the knob. Press the knob and turn it again to set the RCF to "2000". Confirm entry by pressing the knob.**
4. **Select "Time" by turning the knob. Press once and turn the knob again to set the time required. Confirm entry by pressing the knob.**
5. **Open the lid, place the 96-well plates with metal carriers in the buckets, and close the lid.**

   The start and lid keys light up.
6. **Push "Start" to start the centrifuge.**

   When the centrifuge is running the lid key will not be lit. Each run can be interrupted by pushing "Stop".
7. **At the end of the run, the lid key will light up. Open the centrifuge lid by pressing the lid key. Remove the plates.**

   All preset parameters remain in memory after a run has finished.

Warning: Do not centrifuge the plate carriers without the kit plates. If unsupported, the carriers will collapse under high g-forces. Remove the carriers during test runs. Standard microtiter plates may be centrifuged in the same carriers if the g-force does not exceed 500 x *g*.

Preparation of balance plates

When processing one plate at a time, it will be necessary to prepare a balance plate throughout the entire process, including the heating step.

Balance plates should not be used for subsequent purification of DNA from samples: balance plates should only be used to prepare a balance during DNA purification with the Generation Capture Plate purification protocol.

1. **Place the Capture Plate on top of the Waste Plate.**
2. **To prepare a balance for the first centrifugation, add 600 µl water to each well of the Capture Plate.**

   The first centrifugation step is step 4 in the whole blood and bone marrow protocol on page 15, the buffy coat protocol on page 17, the body fluids protocol on page 19, and the Gram-negative bacteria protocol on page 24, and step 5 in the cultured cells and cell suspensions protocol on page 22.

   The balance plates are now ready for centrifugation. Be sure that the Capture Plate is always placed on top of the Waste Plate for centrifugation.

   If centrifuge is not balanced, compare weight of sample plates to weight of balance plates and adjust balance plates by adding or removing water.
3. **To prepare a balance for the second centrifugation, add 400 µl water to each well of the Capture Plate.**

   The second centrifugation step is step 6 in the whole blood and bone marrow protocol on page 15, the buffy coat protocol on page 17, the body fluids protocol on page 19, and the Gram-negative bacteria protocol on page 24, and step 7 in the cultured cells and cell suspensions protocol on page 22.

   The balance plate is ready for centrifugation. Be sure that the Capture Plate is placed on top the Waste Plate for centrifugation.

   If centrifuge is not balanced, compare weight of sample plates to weight of balance plates and adjust balance plates by adding or removing water.
4. **To prepare the balance plate for the third centrifugation, add 200 µl water to the Capture Plate.**

   The third centrifugation step is step 8 in the whole blood and bone marrow protocol on page 15, the buffy coat protocol on page 17, the body fluids protocol on page 19, and the Gram-negative bacteria protocol on page 24, and step 9 in the cultured cells and cell suspensions protocol on page 22.

   The balance plate is ready for centrifugation. Be sure that the Capture Plate is placed on top of the Waste Plate for centrifugation.

   If centrifuge is not balanced, compare weight of sample plates to weight of balance plates and adjust balance plates by adding or removing water.

5. **To prepare the balance plate for the heating step and final centrifugation, first place the Capture Plate on top of the Sample Collection Plate. Add 200 µl water (or a volume equal to the amount of DNA Elution Solution 2 used to elute the DNA samples) to each well of the Capture Plate and seal the plate with a Cap Mat.**

   The heating and final centrifugation step is step 12 in the whole blood and bone marrow protocol on page 16, the buffy coat protocol on page 18, the body fluids protocol on page 20, and the Gram-negative bacteria protocol on page 25, and step 13 in the cultured cells and cell suspensions protocol on page 23.

   The balance plate is ready for heating and centrifugation. Be sure that the Capture Plate is placed on top of the Sample Collection Plate for both the heating and centrifugation steps.

   If centrifuge is not balanced, compare weight of sample plates to weight of balance plates and adjust balance plates by adding or removing water.

Protocol: DNA Purification from Whole Blood and Bone Marrow

Important points before starting

- Use of a multichannel pipet is recommended.

Things to do before starting

- When processing one plate at a time, it will be necessary to prepare a balance plate throughout the entire process, including the heating step. For instructions, see "Preparation of balance plates" on page13.
- 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. **Remove the Plate Cover from the Capture Plate and place the Capture Plate on top of the Waste Plate.**
2. **Add 200 µl well-mixed sample to each well of the Capture Plate by gently touching the center of the matrix with the pipet tip before dispensing.**

   Do not incubate more than 1 h.
3. **Add 400 µl DNA Purification Solution 1.**
4. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**
5. **Remove the Plate Cover and add an additional 400 µl DNA Purification Solution 1.**
6. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**
7. **Remove the Plate Cover and add 200 µl DNA Elution Solution 2.**
8. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**
9. **Remove the Plate Cover from the Sample Collection Plate. Transfer the Capture Plate to the Sample Collection Plate and remove the Plate Cover from the Capture Plate. Discard the Waste Plate.**
10. **Add 200 µl DNA Elution Solution 2 and cover the Capture Plate with a clean Cap Mat.**

    When purifying DNA from one plate of samples, add 200 µl water to the balance plate and cover with a clean Cap Mat.

11. **Heat the Capture Plate and the Sample Collection Plate (and balance plates, if used) in a microwave oven using the recommended microwave settings in the table below.**

Microwave oven model number*	Power setting	Time
Sharp® R-310E	20%	26 min for 2 plates
Sharp R-310FQ	20%	26 min for 2 plates
Sharp R-310HQ	20%	26 min for 2 plates
LG LRM1250	20%	26 min for 2 plates
Panasonic® NN-CT756	100 W	26 min for 2 plates

* Contact QIAGEN Technical Services for recommendations for models not listed.

WARNING  Do not use higher power settings. Higher settings can cause the plates to melt and produce hazardous fumes.

Plates will be hot after heating in the microwave oven. Use protective equipment, such as hot hands or mitts, when removing the plates from the microwave oven.

12. **Centrifuge 3 min at 2000 x *g* immediately following the heating step to collect the purified DNA in the Sample Collection Plate. Do not allow plates to cool.**

For storage, cover the Sample Collection Plate with a clean Cap Mat and store at 4°C. Before use, centrifuge the covered Sample Collection Plate for 3 min at 2000 x *g* to collect condensation at bottom of the wells.

Protocol: DNA Purification from Buffy Coat

Important points before starting

- Use of a multichannel pipet is recommended.

Things to do before starting

- When processing one plate at a time, it will be necessary to prepare a balance plate throughout the entire process, including the heating step. For instructions, see "Preparation of balance plates" on page 13.
- 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 minutes 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 µl buffy coat preparation containing a maximum of 1 x 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. **Remove the Plate Cover from the Capture Plate and place the Capture Plate on top of the Waste Plate.**
2. **Add 200 µl well-mixed sample to each well of the Capture Plate by gently touching the center of the matrix with the pipet tip before dispensing.**

   Incubate no more than 1 h.
3. **Add 400 µl DNA Purification Solution 1.**
4. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**
5. **Remove the Plate Cover and add an additional 400 µl DNA Purification Solution 1.**
6. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**
7. **Remove the Plate Cover and add 200 µl DNA Elution Solution 2.**
8. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**

9. **Remove the Plate Cover from the Sample Collection Plate. Transfer the Capture Plate to the Sample Collection Plate and remove the Plate Cover from the Capture Plate. Discard the Waste Plate.**

10. **Add 200 µl DNA Elution Solution 2 and cover the Capture Plate with a clean Cap Mat.**

    When purifying DNA from one plate of samples, add 200 µl water to the balance plate and cover with a clean Cap Mat.

11. **Heat the Capture Plate and the Sample Collection Plate (and balance plates, if used) in a microwave oven using the recommended microwave settings in the table below.**

Microwave oven model number*	Power setting	Time
Sharp R-310E	20%	26 min for 2 plates
Sharp R-310FQ	20%	26 min for 2 plates
Sharp R-310HQ	20%	26 min for 2 plates
LG LRM1250	20%	26 min for 2 plates
Panasonic NN-CT756	100 W	26 min for 2 plates

* Contact QIAGEN Technical Services for recommendations for models not listed.

WARNING  Do not use higher power settings. Higher settings can cause the plates to melt and produce hazardous fumes.

Plates will be hot after heating in the microwave oven. Use protective equipment, such as hot hands or mitts, when removing the plates from the microwave oven.

12. **Centrifuge 3 min at 2000 x *g* immediately following the heating step to collect the purified DNA in the Sample Collection Plate. Do not allow plates to cool.**

    For storage, cover the Sample Collection Plate with a clean Cap Mat and store at 4°C. Before use, centrifuge the covered Sample Collection Plate for 3 min at 2000 x *g* to collect condensation at bottom of the wells.

Protocol: DNA Purification from Body Fluids

Important points before starting

- Use of a multichannel pipet is recommended.

Things to do before starting

- When processing one plate at a time, it will be necessary to prepare a balance plate throughout the entire process, including the heating step. For instructions, see "Preparation of balance plates" on page 13.
- 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 µl 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. **Remove the Plate Cover from the Capture Plate and place the Capture Plate on top of the Waste Plate.**
2. **Add 200 µl well-mixed sample to each well of the Capture Plate by gently touching the center of the matrix with the pipet tip before dispensing.**

   Incubate no more than 1 h.
3. **Add 400 µl DNA Purification Solution 1.**
4. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**
5. **Remove the Plate Cover and add an additional 400 µl DNA Purification Solution 1.**
6. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**
7. **Remove the Plate Cover and add 200 µl DNA Elution Solution 2.**
8. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**
9. **Remove the Plate Cover from the Sample Collection Plate. Transfer the Capture Plate to the Sample Collection Plate and remove the Plate Cover from the Capture Plate. Discard the Waste Plate.**

10. **Add 200 µl DNA Elution Solution 2 and cover the Capture Plate with a clean Cap Mat.**

    When purifying DNA from one plate of samples, add 200 µl water to the balance plate and cover with a clean Cap Mat.

11. **Heat the Capture Plate and the Sample Collection Plate (and balance plates, if used) in a microwave oven using the recommended microwave settings in the table below.**

Microwave oven model number*	Power setting	Time
Sharp R-310E	20%	26 min for 2 plates
Sharp R-310FQ	20%	26 min for 2 plates
Sharp R-310HQ	20%	26 min for 2 plates
LG LRM1250	20%	26 min for 2 plates
Panasonic NN-CT756	100 W	26 min for 2 plates

* Contact QIAGEN Technical Services for recommendations for models not listed.

WARNING  Do not use higher power settings. Higher settings can cause the plates to melt and produce hazardous fumes.

Plates will be hot after heating in the microwave oven. Use protective equipment, such as hot hands or mitts, when removing the plates from the microwave oven.

12. **Centrifuge 3 min at 2000 x *g* immediately following the heating step to collect the purified DNA in the Sample Collection Plate. Do not allow plates to cool.**

    For storage, cover the Sample Collection Plate with a clean Cap Mat and store at 4°C. Before use, centrifuge the covered Sample Collection Plate for 3 min at 2000 x *g* to collect condensation at bottom of the wells.

Protocol: DNA Purification from Cultured Cells and Cell Suspensions

Important points before starting

- Use of a multichannel pipet is recommended.

Things to do before starting

- When processing one plate at a time, it will be necessary to prepare a balance plate throughout the entire process, including the heating step. For instructions, see "Preparation of balance plates" on page 13.
- 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 µl suspension containing up to 1 x 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 x *g* for 5 seconds. Remove the supernatant, leaving 200 µl 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.
- 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 µl cold PBS (preferably containing 1 mM EDTA to reduce DNase activity; see "Equipment and Reagents to Be Supplied by User", page 9). 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 seconds at the lowest speed <2000 x *g*. Carefully remove the upper 200 µl 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 x 10^7 cells: Determine the number of cells. Centrifuge the appropriate number of cells for 5 min at 300 x *g* in a 1.5 ml microcentrifuge tube. Remove the supernatant leaving behind 200 µl 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 x 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 x 10^7 cells) to a 1.5 ml microcentrifuge tube. Centrifuge for 5 min at 300 x *g*. Remove the supernatant leaving behind 200 µl 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 x 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 µl 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. Remove the Plate Cover from the Capture Plate and place the Capture Plate on top of the Waste Plate.

3. Add 200 µl well-mixed sample to each well of the Capture Plate by gently touching the center of the matrix with the pipet tip before dispensing.

Incubate no more than 1 h.

4. Add 400 µl DNA Purification Solution 1.

5. Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.

6. Remove the Plate Cover and add an additional 400 µl DNA Purification Solution 1.

7. Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.

8. Remove the Plate Cover and add 200 µl DNA Elution Solution 2.

9. Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.

10. Remove the Plate Cover from the Sample Collection Plate. Transfer the Capture Plate to the Sample Collection Plate and remove the Plate Cover from the Capture Plate. Discard the Waste Plate.

* 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.

11. **Add 200 µl DNA Elution Solution 2 and cover the Capture Plate with a clean Cap Mat.**

    When purifying DNA from one plate of samples, add 200 µl water to the balance plate and cover with a clean Cap Mat.

12. **Heat the Capture Plate and the Sample Collection Plate (and balance plates, if used) in a microwave oven using the recommended microwave settings in the table below.**

Microwave oven model number*	Power setting	Time
Sharp R-310E	20%	26 min for 2 plates
Sharp R-310FQ	20%	26 min for 2 plates
Sharp R-310HQ	20%	26 min for 2 plates
LG LRM1250	20%	26 min for 2 plates
Panasonic NN-CT756	100 W	26 min for 2 plates

* Contact QIAGEN Technical Services for recommendations for models not listed.

WARNING Do not use higher power settings. Higher settings can cause the plates to melt and produce hazardous fumes.

Plates will be hot after heating in the microwave oven. Use protective equipment, such as hot hands or mitts, when removing the plates from the microwave oven.

13. **Centrifuge 3 min at 2000 x *g* immediately following the heating step to collect the purified DNA in the Sample Collection Plate. Do not allow plates to cool.**

    For storage, cover the Sample Collection Plate with a clean Cap Mat and store at 4°C. Before use, centrifuge the covered Sample Collection Plate for 3 min at 2000 x *g* to collect condensation at bottom of the wells.

Protocol: DNA Purification from Gram-Negative Bacteria

Important points before starting

- Use of a multichannel pipet is recommended.

Things to do before starting

- When processing one plate at a time, it will be necessary to prepare a balance plate throughout the entire process, including the heating step. For instructions, see "Preparation of balance plates" on page 13.
- Gram-negative bacterial cultures can be used either fresh or frozen. Typically, an overnight culture contains 1–3 x 10^9 cells/ml. Due to the small genome size of Gram-negative bacteria, up to 3 x 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 x *g* for 1 min. Remove the supernatant, leaving 200 µl 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 bacterial samples should be thawed and equilibrated to room temperature (15–25°C) before beginning the procedure.

Procedure

1. **Remove the Plate Cover from the Capture Plate and place the Capture Plate on top of the Waste Plate.**
2. **Add 200 µl well-mixed sample to each well of the Capture Plate by gently touching the center of the matrix with the pipet tip before dispensing.**

   Incubate no more than 1 h.
3. **Add 400 µl DNA Purification Solution 1.**
4. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**
5. **Remove the Plate Cover and add an additional 400 µl DNA Purification Solution 1.**
6. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**
7. **Remove the Plate Cover and add 200 µl DNA Elution Solution 2.**
8. **Cover the Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*.**

9. **Remove the Plate Cover from the Sample Collection Plate. Transfer the Capture Plate to the Sample Collection Plate and remove the Plate Cover from the Capture Plate. Discard the Waste Plate.**

10. **Add 200 µl DNA Elution Solution 2 and cover the Capture Plate with a clean Cap Mat.**

    When purifying DNA from one plate of samples, add 200 µl water to the balance plate and cover with a clean Cap Mat.

11. **Heat the Capture Plate and the Sample Collection Plate (and balance plates, if used) in a microwave oven using the recommended microwave settings in the table below.**

Microwave oven model number*	Power setting	Time
Sharp R-310E	20%	26 min for 2 plates
Sharp R-310FQ	20%	26 min for 2 plates
Sharp R-310HQ	20%	26 min for 2 plates
LG LRM1250	20%	26 min for 2 plates
Panasonic NN-CT756	100 W	26 min for 2 plates

* Contact QIAGEN Technical Services for recommendations for models not listed.

WARNING  Do not use higher power settings. Higher settings can cause the plates to melt and produce hazardous fumes.

Plates will be hot after heating in the microwave oven. Use protective equipment, such as hot hands or mitts, when removing the plates from the microwave oven.

12. **Centrifuge 3 min at 2000 x *g* immediately following the heating step to collect the purified DNA in the Sample Collection Plate. Do not allow plates to cool.**

    For storage, cover the Sample Collection Plate with a clean Cap Mat and store at 4°C. Before use, centrifuge the covered Sample Collection Plate for 3 min at 2000 x *g* to collect condensation at bottom of the wells.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx . 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 sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
Low DNA yield		
a)	Capture Plate allowed to cool before centrifugation to elute the DNA	Samples must be centrifuged while warm to obtain the maximum DNA yield.
b)	Not enough cells in the starting material	If possible, count the cells prior to adding sample to a Capture Plate 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.
c)	DNA Purification Solution 1 added instead of DNA Elution Solution 2 during the elution step	DNA Purification Solution 1 will not release DNA from Capture Plates. Recover DNA as follows: ■ Centrifuge Capture Plate at 2000 x *g* to collect the DNA Purification Solution 1 in a Sample Collection Plate and discard the DNA Purification Solution. ■ Cover Capture Plate with a clean Plate Cover and centrifuge 3 min at 2000 x *g*. ■ Elute DNA by continuing with step 10 on page 15 for the whole blood and bone marrow protocol, page 18 for the buffy coat protocol, page 20 for the body fluids protocol, or page 25 for the Gram-negative bacteria protocol with or step 11 on page 23 for the cultured cells and cell suspensions protocol.

		Comments and suggestions
d)	Plate not heated correctly or microwave setting incorrect	Contact QIAGEN Technical Service (see back cover) for important heating instructions.
Little or no DNA in eluate		
	Plate incorrectly heated	Contact QIAGEN Technical Service (see back cover) for important heating instructions.
Low A_{260}/A_{280} ratio for purified DNA		
a)	Capture Plate overloaded	Capture Plates are designed to purify DNA from up to 1 x 10^7 cells in a 200 µl sample volume. Adding more than 1 x 10^7 cells or sample volumes greater than 200 µl may overload the purification chemistry inhibiting complete cell lysis and resulting in both low yield and low A_{260}/A_{280}.
b)	DNA purified from a sample that was stored improperly	This may lead to a reduced A_{260}/A_{280} ratio, but 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.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
Generation Capture Plate Kit — for quick purification of DNA in 96-well format for PCR amplification		
Generation Capture Plate Kit (12)	For 12 x 96 preps: 12 Capture Plate Sets, 36 Plate Covers, 24 Cap Mats, and reagents	159932
Accessories		
Generation DNA Purif. Solution (1000 ml)	1000 ml DNA Purification Solution 1	159992
Generation DNA Elution Solution (500 ml)	500 ml DNA Elution Solution 2	159994
QIAGEN 96-Well-Plate Centrifugation System		
Centrifuge 4-15C	Universal laboratory centrifuge with brushless motor:	
	100 V, 50/60 Hz	81000
	120 V, 60 Hz	81010
	220 V, 50 Hz	81020
Centrifuge 4K15C	Refrigerated universal laboratory centrifuge with brushless motor:	
	100 V, 50/60 Hz	81200
	220 V, 60 Hz	81210
	220 V, 50 Hz	81220
Plate Rotor 2 x 96*	Rotor for 2 QIAGEN 96 plates, for use with Centrifuge 4-15C or 4K15C	81031

* The Plate Rotor 2 x 96 is available exclusively from QIAGEN and its distributors. Under the current liability and warranty conditions, the rotor may only be used in Centrifuges 4-15, 4-15C, and 4K15C from QIAGEN and freely programmable models of centrifuges 4-15, 4K15, 6-10, 6K10, 6-15, and 6K15 from Sigma Laborzentrifugen GmbH.

Ordering Information

Product	Contents	Cat. no.
Generation Capture Column Kit — for quick purification of DNA for PCR amplification		
Generation Capture Column Kit (50)	For 50 preps: 50 Generation Capture Columns, 50 DNA Collection Tubes, 100 Waste Collection Tubes, and reagents	159914
Generation Capture Column Kit (300)	For 300 preps: 300 Generation Capture Columns, 300 DNA Collection Tubes, 600 Waste Collection Tubes, and reagents	159916

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1063034 04/2010

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