gDNA Extraction Kit

Part Numbers

G7505A
G7505B
SureSelect^{XT} Target Enrichment Kits

Protocol

Version C0, June 2015

For Research Use Only. Not for use in diagnostic procedures.

Agilent Technologies

Notices

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In this Guide...

This document describes how to extract genomic DNA from samples of whole blood, solid tissues and cultured cells using the Agilent SureSelect gDNA Extraction Kit.

If you have comments about this protocol, send an e-mail to techservices@agilent.com.

1 Before You Begin

Make sure you read and understand the information in this chapter and have the necessary equipment and reagents listed before you start an experiment.

2 Procedures

This chapter contains information on how to extract genomic DNA from whole blood, solid tissues and cultured cells. It also contains troubleshooting information.

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Make sure you read and understand the information in this chapter and have the necessary equipment and reagents listed before you start an experiment.

Overview

The gDNA Extraction Kit provides a simple, nontoxic method for efficiently isolating high-molecular-weight genomic DNA from tissue, whole blood and cultured cells. Depending on the starting material, the entire extraction takes only 1.5 to 3 hours to complete and does not require phenol or chloroform. DNA isolated with the DNA Extraction Kit is free from contaminants and may be used to create a DNA sequencing library using Agilent's SureSelect Library Prep Kits.

The gDNA Extraction Kit is a modification of a procedure based on separating contaminating protein from DNA by salt precipitation[1]. The procedure involves digestion of cellular proteins, subsequent removal of the proteins by "salting out" with sodium chloride, precipitation of the DNA with isopropanol and resuspension in the buffer of choice. The number of samples that may be processed simultaneously using this technique is limited only by the centrifuge space available.

Procedural Notes

The yield of genomic DNA obtained with the gDNA extraction kit depends on the type and quantity of starting material. Table 1 lists the expected yields for each source material.

Table 1 gDNA Extraction Kit Specifications

Source Material	Starting Quantity	Yield	Size (kb)
Whole blood	1 ml	3–25 µg (>25 ng/µl)	50–100
Solid tissue	20 mg	3–25 µg (>25 ng/µl)	50–100
Tissue cultured cells	2×10^6 cells	3–25 µg (>25 ng/µl)	50–100

[1] Miller, S. A., Dykes, D. D. and Polesky, H. F. (1998) *Nucleic Acids Res* 16(3): 1215.

Kit Contents

Table 2 Kit Contents - Components Shipped at Room Temperature

Materials Provided	5 extractions/kit Box # 5500-0037	10 extractions/kit Box # 5500-0038	50 extractions/kit Box # 5500-0039	250 extractions/kit Box # 5500-0040
Solution 1 (3×) [a]	20 ml	20 ml	200 ml	500 ml
Solution 2	2.5 ml	2 × 2.5 ml	25 ml	125 ml
Solution 3 [b]	1 ml	2 × 1 ml	10 ml	50 ml

[a] Dilute concentration of Solution 1 to 1× using sterile, deionized water.

[b] Solution 3 is a saturated solution of NaCl, and some precipitation may occur during storage. This precipitation is normal and will not affect DNA extraction.

Table 3 Kit Contents - Components Shipped at –20°C

Materials Provided	5 extractions/kit Box # 5500-0041	10 extractions/kit Box # 5500-0042	50 extractions/kit Box # 5500-0043	250 extractions/kit Box # 5500-0044
Proteinase K	100 µl	2 × 100 µl	1 ml	5 × 1 ml
RNase A	50 µl	2 × 50µl	500 µl	5 × 500 µl

Storage Conditions

Store the Proteinase K and RNase A at –20°C. Store Solutions 1, 2 and 3 at room temperature.

Catalog Information

The gDNA Extraction Kit is included in Agilent's SureSelect^{XT} Kits. It can also be ordered separately using the part numbers listed in Table 4.

Table 4 Reorder Part Numbers

Part Number	Description
G7505A	SureSelect gDNA Extraction Kit, 50 Reactions
G7505B	SureSelect gDNA Extraction Kit, 250 Reactions

Required Equipment and Supplies

Table 5 Required Equipment

Description
Microcentrifuge
Microcentrifuge tubes (1.7-ml)
100% Isopropanol
70% Ethanol
TE buffer (10 mM Tris and 0.1 mM EDTA, pH 8.0)
Sterile, deionized water

2 Procedures

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This chapter contains information on how to extract genomic DNA from whole blood, solid tissues and cultured cells. It also contains troubleshooting information.

Protocol I: Extraction from Whole Blood

Minimum Sample Volume: 1 ml of Whole Blood

Fresh blood extractions yield >3 µg of DNA per ml.

NOTE

Solution 1 is provided at a 3× concentration. Dilute it to 1× using sterile, deionized water.

The protocol below is for 1 ml of whole blood. To scale up the protocol for larger volumes, increase the volumes of the other reagents accordingly.

1. Add 4 ml of Solution 1 (at a 1× concentration) to 1 ml of whole blood in 15-ml centrifuge tube.
2. Incubate the sample on ice for 2 minutes.
3. Spin the sample at 4°C for 10 minutes at 350 × *g* (1500 rpm using a Beckman JS-5.2 rotor or equivalent). Remove as much of the supernatant as possible without disturbing the loose pellet.
4. Resuspend the pellet in the remaining volume of liquid by vortexing for several seconds. Ensure the pellet has been broken up and resuspended before proceeding to step 5.
5. Add 20 µl of Proteinase K (20 mg/ml stock) and 0.5 ml of Solution 2. Briefly vortex to mix then transfer the entire contents to a 1.7-ml microcentrifuge tube.
6. Incubate at 60°C for 10–15 minutes with shaking (e.g. on an Eppendorf 5436 Thermo-mixer at maximum shaker setting). If a thermomixer is unavailable, incubate the sample at 60°C for 15 minutes and vortex it for 5 seconds every 5 minutes.
7. Add 0.2 ml of Solution 3. Invert the tube several times to mix. (*A salt precipitate may be visible in Solution 3. This precipitation is normal and will not affect DNA extraction.*)
8. Chill the sample on ice for another 5 minutes or until a white precipitate appears in the tube.
9. Spin the tube in a microcentrifuge for 15 minutes at 18,000 × *g* at 4°C.
10. Using a large-bore pipet, carefully transfer the supernatant to a fresh 1.7-ml microcentrifuge tube. (Avoid removing any precipitated material when transferring the supernatant.)

11 Add 10 µl of RNase (10 mg/ml stock) to the supernatant and incubate at 37°C for 15 minutes.

NOTE If the mixture appears turbid after this 15-minute incubation, spin the sample for another 10 minutes at 4°C and once again transfer the supernatant to a fresh 1.7-ml tube before proceeding to step 12.

12 Precipitate the DNA by adding an equal volume of 100% isopropanol. Gently invert the tube until the DNA precipitates (strands of a white material will form).

13 Spin the tube in a microcentrifuge for 5 minutes at 18,000 × *g* at 4°C to collect the DNA. Remove the supernatant without disturbing the pellet.

14 Add 0.5 ml of 70% ethanol. Spin again for 5 minutes at 18,000 × *g* at 4°C, then carefully remove the supernatant.

15 Repeat step 14 for a 2nd ethanol wash.

16 Allow the DNA pellet to air dry at room temperature just until the liquid has nearly evaporated. *Take care not to over-dry the pellet.*

17 Resuspend the DNA in 150 µl of TE buffer (10 mM Tris, 0.1 mM EDTA) or your buffer of choice. To dissolve the DNA, incubate the sample at 60–65° C for 15 minutes with shaking (e.g. on an Eppendorf 5436 Thermo-mixer at maximum shaker setting). If a thermomixer is unavailable, incubate the sample at 60–65°C for 15 minutes and vortex it for 5 seconds every 5 minutes.

18 To calculate yield and concentration of your DNA sample, use the equation: 1 OD_{260} = 50 µg/ml.

Protocol II: Extraction from Solid Tissue

Minimum Sample Size: 20 mg of Tissue

Solid tissue extractions yield >3 µg of DNA per 20 mg of tissue.

NOTE Keep tissue on dry ice before adding Solution 2.

1. Weigh out a sample of tissue between 20 and 40 mg. When possible, avoid using a fatty section of the tissue.
2. Cut the sample into small pieces (10 or more pieces for a 20 mg sample) and place the pieces in a 1.7-ml microcentrifuge tube.
3. Optional: To help break up the tissue, crush the sample pieces using a clean glass rod (similar to a mortar and pestle technique).
4. Add 330 µl of Solution 2 and 20 µl of Proteinase K to the tissue sample. Mix thoroughly on a vortex to ensure pieces are well dispersed.
5. Incubate the sample at 60° C for 2 hours with shaking (e.g. on an Eppendorf 5436 Thermo-mixer at maximum shaker setting). If a thermomixer is unavailable, vortex the sample every 30 minutes during the 2 hour incubation at 60°C or incubate the sample at 37°C overnight.
6. Chill the sample on ice for 5 minutes.
7. Add 120 µl of Solution 3. Invert the tube several times to mix. (*A salt precipitate may be visible in Solution 3. This precipitation is normal and will not affect DNA extraction.*)
8. Chill the sample on ice for another 5–10 minutes until a white precipitate appears in the tube.
9. Spin the sample in a microcentrifuge for 15 minutes at 18,000 × *g* at 4°C.
10. Using a large-bore pipet, carefully transfer the supernatant to a fresh 1.7-ml microcentrifuge tube. (Avoid removing any precipitated material when transferring the supernatant.)
11. Add 10 µl of RNase (10 mg/ml stock) to the supernatant and incubate at 37°C for 15 minutes.

NOTE If the mixture appears turbid after this 15-minute incubation, spin the sample for another 10 minutes at 4°C and once again transfer the supernatant to a fresh 1.7-ml tube before proceeding to step 12.

12 Precipitate the DNA by adding an equal volume of 100% isopropanol. Gently invert the tube until the DNA precipitates (strands of a white material will form).

13 Spin the tube in a microcentrifuge for 5 minutes at 18,000 × *g* at 4°C to collect the DNA. Remove the supernatant without disturbing the pellet.

14 Add 0.5 ml of 70% ethanol. Spin again for 5 minutes at 18,000 × *g* at 4°C, then carefully remove the supernatant.

15 Repeat step 14 for a 2nd ethanol wash.

16 Allow the DNA pellet to air dry at room temperature just until the liquid has nearly evaporated. *Take care not to over-dry the pellet.*

17 Resuspend the DNA in 100 µl of TE buffer (10 mM Tris, 0.1 mM EDTA). To dissolve the DNA, incubate the sample at 60–65° C for 15 minutes with shaking (e.g. on an Eppendorf 5436 Thermo-mixer at maximum shaker setting). If a thermomixer is unavailable, incubate the sample at 60–65°C for 15 minutes and vortex it for 5 seconds every 5 minutes.

18 To calculate yield and concentration of your DNA sample, use the equation: 1 OD_{260} = 50 µg/ml.

Protocol III: Extraction from Cultured Cells

Minimum Sample Size: 2 x 10^6 Cells per Extraction

Cultured cell extractions yield >3 µg of DNA per 2,000,000 cells.

NOTE The protocol below is for 2 × 10^6 cultured cells. To scale up the protocol for more cells, increase the volumes of the other reagents accordingly.

1. Harvest the cells from the culture vessel:
   - For suspension cells, decant the cells into 1 or more 50-ml centrifuge tubes. Spin the cells at 100 × *g* for 5 minutes. Remove the supernatant. Resuspend the cells in 10 ml of PBS and determine the concentration using a hemocytometer. Transfer 2 × 10^6 cells to a 1.7-ml microcentrifuge tube.
   - For adherent cells, wash them with 10 ml of PBS and then decant the liquid. Add 2 ml of 0.5% trypsin-EDTA to each flask and incubate at 37°C until cells have detached from the bottom of the flask (typically 2–5 minutes). Spin the cells at 100 × *g* for 5 minutes. Remove the supernatant. Resuspend the cells in 10 ml of PBS and determine the concentration using a hemocytometer. Transfer 2 × 10^6 cells to a 1.7-ml microcentrifuge tube.
2. Add 330 µl of Solution 2 and 20 µl of Proteinase K to the tube of cells. Mix thoroughly on a vortex.
3. Incubate the sample at 60° C for 15 minutes with shaking (e.g. on an Eppendorf 5436 Thermo-mixer at maximum shaker setting). If a thermomixer is unavailable, incubate the sample at 60°C for 15 minutes and vortex it for 5 seconds every 5 minutes.
4. Chill on ice for 5 minutes.
5. Add 120 µl of Solution 3. Invert the tube several times to mix. (*A salt precipitate may be visible in Solution 3. This precipitation is normal and will not affect DNA extraction.*)
6. Chill the sample on ice for another 5–10 minutes until a white precipitate appears in the tube.
7. Spin the sample in a microcentrifuge for 15 minutes at 18,000 × *g* at 4°C.

8 Using a large-bore pipet, carefully transfer the supernatant to a fresh 1.7-ml microcentrifuge tube. (Avoid removing any precipitated material when transferring the supernatant.)

9 Add 10 µl of RNase (10 mg/ml stock) to the supernatant and incubate at 37°C for 15 minutes.

NOTE If the mixture appears turbid after this 15-minute incubation, spin the sample for another 10 minutes at 4°C and once again transfer the supernatant to a fresh 1.7-ml tube before proceeding to step 10.

10 Precipitate the DNA by adding an equal volume of 100% isopropanol. Gently invert the tube until the DNA precipitates (strands of a white material will form).

11 Spin the tube in a microcentrifuge for 5 minutes at 18,000 × *g* at 4°C to collect the DNA. Remove the supernatant without disturbing the pellet.

12 Add 0.5 ml of 70% ethanol. Spin again for 5 minutes at 18,000 × *g* at 4°C, then carefully remove the supernatant.

13 Repeat step 12 for a 2nd ethanol wash.

14 Allow the DNA pellet to air dry at room temperature just until the liquid has nearly evaporated. *Take care not to over-dry the pellet.*

15 Resuspend the DNA in 100 µl of TE buffer (10 mM Tris, 0.1 mM EDTA). To dissolve the DNA, incubate the sample at 60–65° C for 15 minutes with shaking (e.g. on an Eppendorf 5436 Thermo-mixer at maximum shaker setting). If a thermomixer is unavailable, incubate the sample at 60–65°C for 15 minutes and vortex it for 5 seconds every 5 minutes.

16 To calculate yield and concentration of your DNA sample, use the equation: 1 OD_{260} = 50 µg/ml.

Troubleshooting

If you get low DNA yield

- ✔ The quality of the starting material may not be optimal. Increase the amount of starting material. When using more than 5 million cells or more than 50 mg of the solid tissue, scale up the volumes of the reagents accordingly.
- ✔ Whenever possible, use starting material that is of good quality and has been properly stored. Tissue samples are best stored at –80°C. For blood and cultured cells, best results are obtained with fresh samples. When using frozen samples, avoid repeated freeze/thaw cycles.
- ✔ For extractions from tissue, ensure the sample is cut into several small pieces to facilitate Proteinase K digestion (see Protocol II, step 2)
- ✔ Be careful not to over dry the pellet of gDNA. An overly dried pellet can be difficult to resuspend.
- ✔ Increase the of incubation time for the Proteinase K digestion or increase the amount of Proteinase K used in the digestion.

If the $A_{260/280}$ ratio is below 1.7

- ✔ Increase the amount of time the proteins are allowed to precipitate in Solution 3.
- ✔ After the RNase digestion, chill the sample tube on ice for 5 minutes then spin in a microcentrifuge for 10 minutes at 18,000 × *g* at 4°C.
- ✔ If the DNA sample appears milky after resuspending the pellet in TE buffer, spin the sample in a microcentrifuge to pellet any remaining proteins (5–10 minutes at 18,000 × *g* at 4°C). After the spin, transfer the supernatant containing the gDNA to a fresh tube and measure the absorbance at 260 and 280 nm again.
- ✔ Ensure all spins are carried out at 4°C. Spinning the sample at room temperature may allow proteins to redissolve into solution.

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In This Book

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