DNA Extraction Kit

INSTRUCTION MANUAL

Catalog #200600

Revision C.0

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

200600-12

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Agilent. Agilent shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

United States and Canada

Agilent Technologies
Stratagene Products Division
11011 North Torrey Pines Road
La Jolla, CA 92037
Telephone (858) 373-6300
Order Toll Free (800) 424-5444
Technical Services (800) 894-1304
Internet techservices@agilent.com
World Wide Web www.stratagene.com

Europe

Location	Telephone	Fax	Technical Services
Austria	0800 292 499	0800 292 496	0800 292 498
Belgium	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 15775	0800 15740	0800 15720
France	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 919 288	0800 919 287	0800 919 289
Germany	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 182 8232	0800 182 8231	0800 182 8234
Netherlands	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 023 0446	+31 (0)20 312 5700	0800 023 0448
Switzerland	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 563 080	0800 563 082	0800 563 081
United Kingdom	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 917 3282	0800 917 3283	0800 917 3281

All Other Countries

Please contact your local distributor. A complete list of distributors is available at www.stratagene.com.

DNA Extraction Kit

CONTENTS

DNA Extraction Kit

MATERIALS PROVIDED

Materials provided[a]	Amount	Storage temperature
Buffers		
Solution 1	500 ml of 3× concentrate[b]	room temperature
Solution 2	420 ml	room temperature
Solution 3[c]	150 ml	room temperature
RNase	1 ml 10 mg/ml stock	–20°C
Pronase	2 ml 225 mg/ml stock	–20°C

[a] The DNA Extraction Kit contains enough reagents to isolate DNA from 35 blood samples (10 ml/sample), 35 tissue culture pellets (1 × 10^8 cells/pellet) or 30 solid tissue samples (250 mg tissue/sample).

[b] Dilute concentrate to 1× final concentration with sterile, deionized water.

[c] 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.

STORAGE CONDITIONS

Enzymes: –20°C
Solutions: Room Temperature

INTRODUCTION

The DNA Extraction Kit provides a simple, nontoxic method for efficiently isolating high-molecular-weight DNA from tissue, whole blood and cultured cells. Depending on the starting material, the entire extraction takes only two to three 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 directly for restriction digests, cloning, Southern blotting, PCR amplification, and other DNA analysis techniques.

The DNA Extraction Kit[1] is a modification of a procedure based on separating contaminating protein from DNA by salt precipitation.[2] The procedure involves digestion of cellular proteins, subsequent removal of the proteins by "salting out" using standard sodium chloride, precipitation of the DNA with ethanol 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.

DNA Extraction Kit Specifications

Source	Quantity	Yield	Size (kb)	Time
Whole blood	5 ml	>30 µg	100–500	2 hours
Whole tissue	1 gm	>250 µg	50–100	2 hours, 45 minutes
Tissue cultured cells	10^8 cells	>600 µg	50–100	2 hours, 15 minutes

Revision C.0

PROTOCOL I: EXTRACTION FROM WHOLE BLOOD

Sample Volume: 5–10 ml of Whole Blood

Fresh blood extractions yield 30–100 µg of DNA. The yield depends on the source and freshness of blood. Lower yields will occur with blood that has been stored for a few days.

1. Add 40–45 ml of 1× Solution 1 (prepared by mixing 15 ml of 3× concentrate and 30 ml of ddH_2O) to the blood sample to yield a final volume of 50 ml.

2. Incubate the sample on ice for 2 minutes.

3. Spin the sample for 15 minutes at 350 × *g* (1500 rpm using a Beckman JS-5.2 rotor) at 4°C. Discard the supernatant and save the pellet; while removing the supernatant, be careful to not discard the gelatinous-appearing pellet.

4. Resuspend the pellet in 11 ml of Solution 2.

5. Add pronase (0.44 µl stock/ml sample) to the suspension to yield a final concentration of 100 µg/ml.

6. Incubate with shaking at 60°C for 1 hour, or at 37°C overnight.

7. Chill the tube on ice for 10 minutes.

8. Add 4 ml of Solution 3 to the tube. Invert several times to mix, then place the sample on ice for 5 minutes.

   Note *A precipitate may be visible in solution 3. This precipitation is normal and will not affect DNA extraction.*

9. Pellet the protein precipitate by spinning for 15 minutes at 2000 × *g* (3400 rpm using a Beckman JS-5.2 rotor) at 4°C.

10. Using a large-bore pipet, carefully transfer the supernatant to a sterile 50-ml conical tube.

    Note *Avoid removing any flocculent material when transferring the supernatant.*

11. Add RNase (2 µl stock/ml sample) to the supernatant to yield a final concentration of 20 µg/ml.

12. Incubate at 37°C for 15 minutes.

13. Precipitate the DNA by adding 2 volumes of 100% ethanol to the supernatant. Gently invert until the DNA precipitates (strands of a white, flocculent material will form).

14. Remove the DNA by spooling with a sterile glass rod.

15. Rinse the DNA while still on the rod with 70% ethanol. Dry the spooled DNA by briefly touching to a Kimwipe.®

16. Carefully resuspend the DNA in 500 µl of 10 mM Tris, 0.1 mM EDTA buffer, by gently inverting the tube. Do not vortex or pipet sample. Store at 4°C.

17. To calculate yield and concentration of your DNA sample, 1 OD_{260} = 50 µg/ml.

    Note *To avoid shearing the genomic DNA, use wide-bore tips during manipulations.*

PROTOCOL II: EXTRACTION FROM WHOLE TISSUE

Sample Size: ~250 mg of Tissue

Whole tissue extractions yield 250–1100 µg of DNA per gram of tissue.

Note *Tissue should be kept on dry ice before adding Solution 2.*

1. Add 14 ml of Solution 2 to the tissue sample.

2. Homogenize the tissue with a Dounce homogenizer or with a mechanical homogenizer at medium setting.

3. Add pronase (0.44 µl stock/ml sample) to homogenate to yield a final concentration of 100 µg/ml.

4. Incubate with shaking at 55°C for 2 hours or 37°C overnight.

5. Chill on ice for 10 minutes.

6. Add 5 ml of Solution 3. Invert several times to mix.

   Note *A precipitate may be visible in solution 3. This precipitation is normal and will not affect DNA extraction.*

7. Incubate on ice for 5 minutes.

8. Pellet the precipitate for 15 minutes at 2000 × *g* (3400 rpm with a Beckman JS-5.2 rotor) at 4°C.

9. Carefully transfer the supernatant with a large-bore pipet to a sterile 50-ml conical tube.

   Note *Avoid removing any flocculent material when transferring the supernatant.*

10. Add RNase (2 µl stock/ml sample) to the supernatant to yield a final concentration of 20 µg/ml.

11. Incubate at 37°C for 15 minutes.

12. Proceed to steps 13–17 in Protocol I.

PROTOCOL III: EXTRACTION FROM CULTURED CELLS

Sample Size: 1 x 10^8 Cells per Extraction

Cultured cell extractions yield 600–1000 µg DNA per 10^8 cells.

1. Harvest the cells from the culture vessel.

2. Pellet the cells at 350 × *g* (1500 rpm with a Beckman JS-5.2 rotor) at 4°C for 15 minutes.

3. Discard the supernatant and resuspend the cells in 20 ml of phosphate buffered saline.

4. Repeat steps 2 and 3.

5. Discard the supernatant. Add 11 ml of Solution 2 to the cell pellet.

6. Homogenize the pellet as described in Protocol II, step 2.

7. Add pronase (0.44 µl stock/ml sample) to the homogenized pellet to yield a final concentration of 100 µg/ml.

8. Incubate with shaking at 60°C for 1 hour or at 37°C overnight.

9. Chill on ice for 10 minutes.

10. Add 4 ml of Solution 3. Invert several times to mix.

    Note *A precipitate may be visible in solution 3. This precipitation is normal and will not affect DNA extraction.*

11. Incubate on ice for 5 minutes.

12. Pellet the precipitate for 15 minutes at 2000 × *g* (3400 rpm with a Beckman JS-5.2 rotor) at 4°C.

13. Carefully transfer the supernatant using a large-bore pipet to a sterile 50-ml conical tube.

    Note *Avoid removing any flocculent material when transferring the supernatant.*

14. Add RNase (2 µl stock/ml sample) to the supernatant to yield a final concentration of 20 µg/ml.

15. Incubate at 37°C for 15 minutes.

16. Proceed to steps 13–17 in Protocol I.

Note *If the DNA does not visibly precipitate upon adding ethanol, place the tube at –20°C for a minimum of 2 hours, or overnight. Then pellet the DNA by spinning at 2000 × g for 20 minutes.*

REFERENCES

1. Grafsky, A. J., Deely, D. and Braman, J. C. (1990) *Strategies* 3(2):27–28.
2. Miller, S. A., Dykes, D. D. and Polesky, H. F. (1988) *Nucleic Acids Res* 16(3):1215.

ENDNOTES

Kimwipes® is a registered trademark of Kimberly-Clark Corporation.

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE

An Agilent Technologies Division

DNA Extraction Kit

QUICK-REFERENCE PROTOCOL

Whole Blood

Steps	Procedure	Time Required
1–3	Wash and concentrate cells	25 minutes
4–7	Lyse cells	75 minutes
8–10	Isolate nucleic acids from proteins	30 minutes
11–16	Purify DNA	30 minutes

Whole Tissue

Steps	Procedure	Time Required
1–2	Homogenize tissue	10 minutes
3–5	Lyse cells	2 hours
6–9	Isolate nucleic acids from proteins	30 minutes
10–12	Purify DNA	30 minutes

Cultured Cells

Steps	Procedure	Time Required
1–3	Concentrate cells	30 minutes
4–5	Wash cells	30 minutes
6–9	Lyse cells	1.5 hours
10–13	Isolate nucleic acids from proteins	30 minutes
14–16	Purify DNA	30 minutes