

# DNeasy<sup>®</sup> Tissue Handbook

For DNA purification from

Animal tissues

Rodent tails

Cultured cells

Bacteria

Yeast

Animal blood

Insects

Fixed tissues

May 2002



© 2002 QIAGEN, all rights reserved.

## QIAGEN Worldwide

---

### QIAGEN Companies

<b>Australia</b>	<b>QIAGEN Pty Ltd</b> ABN 75 072 382 944	PO Box 25 • Clifton Hill • Victoria 3068 Orders 03-9489-3666 • Fax 03-9489-3888 • Technical 1-800-243-066
<b>Canada</b>	<b>QIAGEN Inc.</b>	2800 Argentia Road • Unit 7 • Mississauga • Ontario • L5N 8L2 Orders 800-572-9613 • Fax 800-713-5951 • Technical 800-DNA-PREP (800-362-7737)
<b>France</b>	<b>QIAGEN S.A.</b>	3 avenue du Canada • LP 809 • 91974 COURTABOEUF CEDEX Orders 01-60-920-920 • Fax 01-60-920-925 • Technical 01-60-920-930
<b>Germany</b>	<b>QIAGEN GmbH</b>	Max-Volmer-Straße 4 • 40724 Hilden Orders 02103-29-12000 • Fax 02103-29-22000 • Technical 02103-29-12400
<b>Italy</b>	<b>QIAGEN S.p.A.</b>	Via Grosio, 10/10 • 20151 Milano Orders 02-33430411 • Fax 02-33430426 • Technical 02-33430414
<b>Japan</b> <a href="http://www.qiagen.co.jp">www.qiagen.co.jp</a>	<b>QIAGEN K.K.</b>	Forefront Tower II • 13-1, Kachidoki 3 Chome • Chuo-ku, Tokyo 104-0054 Telephone 03-5547-0811 • Fax 03-5547-0818 • Technical 03-5547-0811
<b>Switzerland</b>	<b>QIAGEN AG</b>	Auf dem Wolf 39 • 4052 Basel Orders 061-319-30-30 • Fax 061-319-30-33 • Technical 061-319-30-31
<b>UK and Ireland</b>	<b>QIAGEN Ltd.</b>	Boundary Court • Gatwick Road • Crawley • West Sussex, RH10 9AX Orders 01293-422-911 • Fax 01293-422-922 • Technical 01293-422-999
<b>USA</b>	<b>QIAGEN Inc.</b>	28159 Avenue Stanford • Valencia • CA 91355 Orders 800-426-8157 • Fax 800-718-2056 • Technical 800-DNA-PREP (800-362-7737)

[www.qiagen.com](http://www.qiagen.com)

### QIAGEN Distributors

Please see the last page for contact information for your local QIAGEN distributor.

# Contents

<b>Kit Contents</b>	<b>4</b>
<b>Storage Conditions</b>	<b>4</b>
<b>Reagents and Equipment to be Supplied by the User</b>	<b>4</b>
<b>Product Use Limitations</b>	<b>5</b>
<b>Product Warranty and Satisfaction Guarantee</b>	<b>5</b>
<b>Technical Assistance</b>	<b>5</b>
<b>Safety Information</b>	<b>6</b>
<b>Introduction</b>	<b>7</b>
<b>The DNeasy Principle and Procedure</b>	<b>7</b>
<b>High-Throughput Sample Processing</b>	<b>7</b>
<b>Technical Information</b>	<b>9</b>
Sample collection and storage	9
Starting amounts of samples	9
Maximum amount of starting material	9
Very small sample sizes	10
Quantification of starting material	11
Proteinase K	12
Copurification of RNA	12
Elution of pure nucleic acids	13
Expected yields	14
Determination of yield and purity	15
Determination of length	16
Purification of high-molecular-weight DNA	16
<b>The DNeasy Protocols</b>	<b>17</b>
■ <b>Animal Tissues</b>	<b>17</b>
■ <b>Rodent Tails</b>	<b>20</b>
■ <b>Cultured Animal Cells</b>	<b>23</b>
<b>Guidelines for Preparation and Lysis of Other Starting Materials</b>	<b>25</b>
Animal blood	25
Fixed tissues	26
Bacteria	27
Yeasts	28
Insects	29
Crude lysates	29
<b>Troubleshooting Guide</b>	<b>30</b>
<b>Selected References</b>	<b>34</b>
<b>Ordering Information</b>	<b>37</b>
<b>QIAGEN Distributors</b>	<b>43</b>

## Kit Contents

DNeasy Tissue Kits		
Catalog No.	69504	69506
Number of preparations	50	250
DNeasy® Mini Columns in 2 ml Collection Tubes	50	250
Collection Tubes (2 ml)	100	500
Buffer ATL	10 ml	50 ml
Buffer AL*	12 ml	54 ml
Buffer AW1* (concentrate)	19 ml	95 ml
Buffer AW2† (concentrate)	13 ml	66 ml
Buffer AE	22 ml	2 x 60 ml
Proteinase K	1.25 ml	6 ml
Handbook	1	1

\* Contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents containing bleach.

† Contains sodium azide as a preservative. Sodium azide is highly toxic and may react explosively with lead or copper drainpipes. Take appropriate safety measures and wear gloves when handling. Flush drains thoroughly with water after disposing of solutions containing sodium azide and follow your institution's waste-disposal guidelines.

## Storage Conditions

DNeasy spin columns and buffers can be stored dry at room temperature (15–25°C) for up to 1 year without showing any reduction in performance.

DNeasy Tissue Kits contain a novel ready-to-use proteinase K solution, which is supplied in a specially formulated storage buffer. Proteinase K is stable for at least one year after delivery when stored at room temperature. For storage longer than one year or if ambient temperatures often exceed 25°C, we suggest storing proteinase K at 2–8°C.

## Equipment and Reagents to be Supplied by the User

- Microcentrifuge tubes for lysis and elution (1.5 ml or 2 ml)
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- PBS (for use with cultured cells only)
- Ethanol (96–100%)

## Product Use Limitations

DNeasy Tissue Kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

## 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. We will credit your account or exchange the product — as you wish.

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 inside front cover).

## 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 DNeasy Tissue 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 inside front cover).

## 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.

**CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.**

The following risk and safety phrases apply to the components of the DNeasy Tissue Kits. Buffers AL and AW1 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

### Buffer AL and Buffer AW1 (concentrate)

Contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases: \* R22-36/38, S13-26-36-46

### Proteinase K

Contains proteinase K: sensitizer, irritant. Risk and safety phrases: \* R36/37/38-42/43 S23-24-26-36/37

### 24-hour emergency information

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

*\* R22: Harmful if swallowed; R36/37/38: Irritating to eyes, respiratory system and skin; R36/38: Irritating to eyes and skin; R42/43: May cause sensitization by inhalation and skin contact; S13: Keep away from food, drink, and animal feedingstuffs; S23: Do not breath spray; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37: Wear suitable protective clothing and gloves; S46: If swallowed, seek medical advice immediately and show this container or label.*

## Introduction

DNeasy Tissue Kits are designed for rapid isolation of total DNA (e.g., genomic, mitochondrial, and viral) from a variety of sample sources including fresh or frozen animal tissues and cells, yeasts, or bacteria. DNeasy purified DNA is free of contaminants and enzyme inhibitors and is highly suited for PCR, Southern blotting, RAPD, AFLP, and RFLP applications.

## The DNeasy Tissue Principle and Procedure

DNeasy Tissue Kits use advanced silica-gel–membrane technology for rapid and efficient purification of total cellular DNA without organic extraction or ethanol precipitation. The buffer system is optimized to allow direct cell lysis followed by selective binding of DNA to the DNeasy membrane. After lysis, the DNeasy procedure can be completed in as little as 20 minutes. Simple centrifugation processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cations, and allows simultaneous processing of multiple samples in parallel. In addition, the DNeasy procedure is suitable for a wide range of sample sizes.

The DNeasy procedure is simple. Samples are first lysed using proteinase K (mechanical homogenization is not necessary). Buffering conditions are adjusted to provide optimal DNA-binding conditions and the lysate is loaded onto the DNeasy mini column. During a brief centrifugation, DNA is selectively bound to the DNeasy membrane as contaminants pass through. Remaining contaminants and enzyme inhibitors are removed in two efficient wash steps and DNA is then eluted in water or buffer, ready for use. DNeasy purified DNA typically has an  $A_{260}/A_{280}$  ratio between 1.7 and 1.9, and is up to 50 kb in size, with fragments of 30 kb predominating. The DNeasy procedure also efficiently recovers DNA fragments as small as 100 bp.

## High-Throughput Sample Processing

For high-throughput needs, QIAGEN offers the DNeasy 96 Tissue Kit — DNeasy technology in a convenient, 96-well format for DNA isolation from rodent tails and other tissues. Please contact Technical Services or your local distributor for more information.

# DNeasy Tissue Procedure

Tissue sample



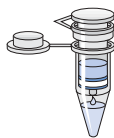
Lyse



Bind DNA



Wash



Elute



Ready-to-use DNA

# Technical Information

## Sample collection and storage

Best results are obtained with fresh material or material that has been immediately frozen and stored at  $-20^{\circ}\text{C}$  or  $-70^{\circ}\text{C}$ . Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor quality starting material will also lead to reduced length and yield of purified DNA.

Tissue samples can also be stored in Buffer ATL after proteinase K digestion for up to 6 months at ambient temperature without any reduction in DNA quality.

For certain bacterial or yeast cultures that accumulate large amounts of metabolites and/or form very dense cell walls, it is preferable to harvest cells in the early log phase of growth. Fresh or frozen cell pellets can be used in the procedure.

## Starting amounts of samples

The DNeasy Tissue procedure gives DNA yields that increase linearly over a wide range, for both very small and large sample sizes (e.g., from as little as 100 cells up to  $5 \times 10^6$  cells).

## Maximum amount of starting material

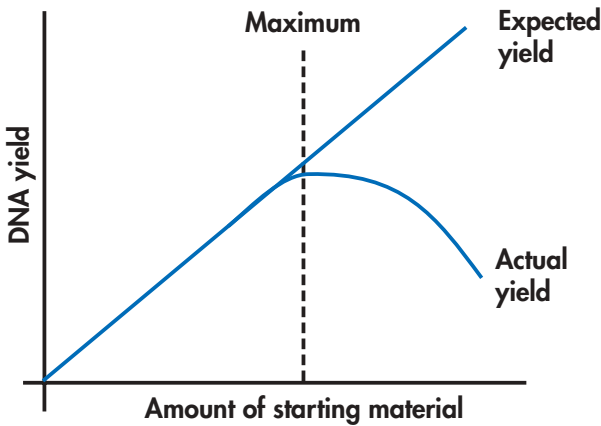
In order to obtain optimum DNA yield and quality, it is important not to overload the DNeasy procedure, as this can lead to significantly lower yields than expected see Figure 1. For samples with very high DNA contents (e.g., spleen, which has a high cell density, and cell lines with a high degree of ploidy), less than the recommended amount of sample listed in Table 1 should be used. If your starting material is not shown in Table 3 (page 14) and you have no information regarding DNA content, we recommend beginning with half the maximum amount of starting material indicated in Table 1. Depending on the yield obtained, the sample size can be increased in subsequent preparations.

**Table 1. Maximum amounts of starting material**

Animal tissue	25 mg
Mouse tail	0.6–1.2 cm
Rat tail	0.6 cm
Cultured cells	$5 \times 10^6$
Bacteria	$2 \times 10^9$
Yeast	$5 \times 10^7$

## Very small sample sizes

The DNeasy procedure is also suitable for purifying DNA from very small amounts of starting material. If the sample has less than 5 ng DNA (<10,000 copies), 3–5 µg carrier DNA (a homopolymer such as poly dA, poly dT, or gDNA) should be added to the starting material. Ensure that the carrier DNA does not interfere with your downstream application. In order to prevent any interference of the carrier with the downstream application, an RNA carrier can be used. This can be removed later by RNase digestion. DNA or RNA homopolymers can be purchased from various suppliers.



**Figure 1.** Schematic diagram of effect of sample size on DNA yield. If more than the maximum amount of starting material is used, DNA yield will be lower than expected.

## Quantification of starting material

Weighing tissue or counting cells is the most accurate way to quantify starting material. However, the approximate guidelines given below can also be followed.

### Animal tissue

2 mm<sup>3</sup> of most animal tissues weighs approximately 10–15 mg.

### Animal cells

The number of HeLa cells obtained in various culture dishes after confluent growth is given in Table 2.

**Table 2. Growth area and number of HeLa cells in various culture dishes**

Cell culture vessel	Growth area* (cm <sup>2</sup> )	Number of cells <sup>†</sup>
<b>Multiwell plates</b>		
96-well	0.32–0.6	4–5 × 10 <sup>4</sup>
48-well	1	1 × 10 <sup>5</sup>
24-well	2	2.5 × 10 <sup>5</sup>
12-well	4	5 × 10 <sup>5</sup>
6-well	9.5	1 × 10 <sup>6</sup>
<b>Dishes</b>		
Ø 35-mm	8	1 × 10 <sup>6</sup>
Ø 60-mm	21	2.5 × 10 <sup>6</sup>
Ø 100-mm	56	7 × 10 <sup>6</sup>
Ø 145–150-mm	145	2 × 10 <sup>7</sup>
<b>Flasks</b>		
40–50-ml	25	3 × 10 <sup>6</sup>
250–300-ml	75	1 × 10 <sup>7</sup>
650–750-ml	162–175	2 × 10 <sup>7</sup>

\* Per well, if multiwell plates are used; varies slightly depending on the supplier.

<sup>†</sup> Cell numbers given are for HeLa cells (approximate length = 15 µm) assuming confluent growth.

Cell numbers vary since animal cells can vary in length from 10–100 µm.

### Bacteria and yeast

Bacterial and yeast growth are usually measured using a spectrophotometer. However, it is very difficult to give specific and reliable recommendations for the relations between OD values and cell numbers in bacterial and yeast cultures. Cell density is influenced by a variety of factors (e.g., species, media, and shaker speed) and OD readings of cultures measure light scattering rather than absorption. Measurements of light scattering are highly dependent on the distance between the sample and the detector and therefore readings vary between different types of spectrophotometer. In addition, different species show different OD values at defined wavelengths (e.g., 600 or 436 nm).

We therefore recommend calibrating the spectrophotometer used by comparing OD measurements at appropriate wavelengths with viable cell densities determined by plating experiments (e.g., see Ausubel, F.M. et al., eds. (1991) *Current Protocols in Molecular Biology*, Wiley Interscience). OD readings should be between 0.05 and 0.3 to ensure significance. Samples with readings above 0.3 should be diluted so that the readings fall within this range and the dilution factor used in calculating the number of cells per ml.

The following calculation can be considered as a rough guide which may be helpful. An *E. coli* culture of  $1 \times 10^9$  cells per ml, diluted 1 in 4, gives OD<sub>600</sub> values of 0.25 measured using a Beckman DU-7400 or 0.125 using a Beckman DU-40 spectrophotometer. These correspond to calculated OD values of 1.0 or 0.5 respectively for  $1 \times 10^9$  cells per ml. The same OD<sub>600</sub> values of 1.0 or 0.5 respectively are obtained for a *S. cerevisiae* culture with a cell density of  $1-2 \times 10^7$  cells per ml.

## Proteinase K

DNeasy Kits contain ready-to-use proteinase K supplied in a specially formulated storage buffer. The concentration of proteinase K as supplied is 20 mg/ml (600 mAU/ml solution, or 40 mAU/mg protein), and has been chosen to provide optimal results.

Also included in the kits is an optimized buffer for tissue lysis, Buffer ATL. The DNeasy procedure does not require mechanical disruption of the sample, but it is advisable to cut animal tissue into small pieces to enable efficient lysis. If desired, lysis time can be reduced to 20 min by grinding the sample in liquid nitrogen before addition of Buffer ATL and proteinase K.

Proteinase K is stable for at least one year after delivery when stored at room temperature (15–25°C). To store for more than one year or if ambient temperature often exceeds 25°C, we suggest keeping proteinase K at 2–8°C.

Please contact Technical Services or your local distributor if you have any questions about the use of proteinase K.

## Copurification of RNA

DNeasy mini columns copurify DNA and RNA when both are present in the sample (see Table 3, page 14). Transcriptionally active tissues such as liver and kidney contain high levels of RNA, which will be copurified. RNA may inhibit some downstream enzymatic reactions, although it does not affect PCR. If RNA-free genomic DNA is required, RNase A should be added to the sample prior to addition of Buffer AL to digest the RNA. DNeasy protocols describe the use of an RNase A stock solution of 100 mg/ml. However, the amounts of RNase can be adjusted with less concentrated stock solutions, but not more than 20 µl of RNase solution should be used. Please refer to the protocols for details.

## Elution of pure nucleic acids

Purified DNA is eluted from the DNeasy mini column in either Buffer AE or water. Typically, elution is performed in two successive steps using 200  $\mu$ l Buffer AE each time. Bear in mind that elution volume and number of elution steps depends on the amount of DNA bound to the DNeasy membrane (see Figure 2).

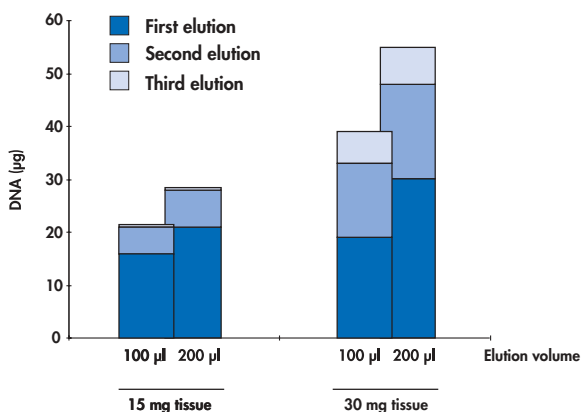
For samples containing up to 10  $\mu$ g DNA, a single elution with 200  $\mu$ l is sufficient. For samples containing more than 10  $\mu$ g, a second elution step with another 200  $\mu$ l Buffer AE is recommended. Approximately 60–80% of the DNA will elute in the first elution. If >30  $\mu$ g is bound to the DNeasy membrane, elution in 3 x 200  $\mu$ l may increase yield (see below).

Elution in 100  $\mu$ l increases the DNA concentration in the eluate, but reduces overall DNA yield. To prevent dilution of the first eluate, the subsequent elution step can be performed using a fresh 1.5 ml microcentrifuge tube. More than 200  $\mu$ l should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.

For very small samples (containing less than 1  $\mu$ g of DNA), only one elution in 50  $\mu$ l of Buffer AE or water is recommended.

Elution with Buffer AE guarantees optimal recovery and stability of eluted DNA. However, if you wish to elute DNA with water please ensure that the pH of the water is at least 7.0 (deionized water from certain sources can be acidic). For long-term storage of DNA, elution in Buffer AE is strongly recommended since DNA stored in water is subject to acid hydrolysis.

Buffer AE should be used at room temperature (15–25°C). Heating Buffer AE before elution is not necessary.



**Figure 2.** Yields of total nucleic acids in successive elutions of 100 or 200  $\mu$ l.

## Expected yields

Yields of genomic DNA will vary from sample to sample depending on the amount and type of material processed. In addition, the quality of starting material will affect DNA yield.

The following can be used to provide an estimate of expected yield:

- Animal tissue: 0.2–1.2 µg of DNA per milligram of tissue
- Mouse tail: 10–40 µg of DNA depending on type, length, and age of tail used
- Mammalian cells: 6 pg of DNA per cell (6 µg per 10<sup>6</sup> cells)

For more details, see Table 3 below.

**Table 3. Yields with DNeasy Tissue Kits**

Source	Yield	
	Total nucleic acids (µg)*	DNA (µg)†
Lymphocytes (5 x 10 <sup>6</sup> )	20–30	15–25
HeLa cells (2 x 10 <sup>6</sup> )	40–60	15–25
Liver (25 mg)	60–115	10–30
Brain (25 mg)	35–60	15–30
Lung (25 mg)	8–20	5–10
Heart (25 mg)	25–45	5–10
Kidney (25 mg)	40–85	15–30
Spleen (10 mg)	25–45	5–30
Mouse tail, 1.2 cm (tip section)	15–30	10–25
Rat tail, 0.6 cm (tip section)	25–60	20–40

\* Nucleic acids obtained without RNase treatment.

† Nucleic acids obtained with RNase treatment.

## Determination of yield and purity

DNA yield is determined by measuring the concentration of DNA in the eluate by its absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.15 and 1.0 to be accurate. Sample dilution should be adjusted accordingly: e.g., an eluate containing 25–50 ng DNA/ $\mu\text{l}$  ( $A_{260} = 0.5\text{--}1.0$ ) should not be diluted with more than 4 volumes of water. Measure the absorbance at 260 nm or scan absorbance from 220–330 nm (a scan will show if there are other factors affecting absorbance at 260 nm; for instance, absorbance at 325 nm would indicate contamination by particulate matter or a dirty cuvette). An  $A_{260}$  of 1 (with a 1 cm detection path) corresponds to 50  $\mu\text{g}$  DNA per milliliter water. Water should be used as diluent when measuring DNA concentration since the relationship between absorbance and concentration is based on extinction coefficients calculated for nucleic acids in water.\* Both DNA and RNA are measured with a spectrophotometer at 260 nm; to measure only DNA in a mixture of DNA and RNA, a fluorimeter must be used.

An example of the calculations involved in DNA quantification is shown below:

Volume of DNA sample	= 100 $\mu\text{l}$
Dilution	= 20 $\mu\text{l}$ of DNA sample + 180 $\mu\text{l}$ distilled water (1/10 dilution)
Measure absorbance of diluted sample in a 0.2 ml cuvette	
$A_{260}$	= 0.2
Concentration of DNA sample	= 50 $\mu\text{g}/\text{ml}$ $\times A_{260}$ $\times$ dilution factor = 50 $\mu\text{g}/\text{ml}$ $\times$ 0.2 $\times$ 10 = 100 $\mu\text{g}/\text{ml}$
Total amount	= concentration $\times$ volume of sample in milliliters = 100 $\mu\text{g}/\text{ml}$ $\times$ 0.1 ml = 10 $\mu\text{g}$ of DNA

The ratio of the readings at 260 nm and 280 nm ( $A_{260}/A_{280}$ ) provides an estimate of the purity of DNA with respect to contaminants that absorb UV, such as protein. However, the  $A_{260}/A_{280}$  ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting  $A_{260}/A_{280}$  ratio can vary greatly. Lower pH results in a lower  $A_{260}/A_{280}$  ratio and reduced sensitivity to protein contamination. For accurate values, we recommend measuring absorbance in 10 mM Tris-Cl, pH 8.5, in which pure DNA has an  $A_{260}/A_{280}$  ratio of 1.8–2.0. Always be sure to calibrate the spectrophotometer with the same solution.

\* Wilfinger, W.W., Mackey, M., and Chomcynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *BioTechniques* **22**, 474.

## Determination of length

The precise length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. The DNA should be concentrated by alcohol precipitation, and redissolved in approximately 30  $\mu$ l TE buffer, pH 8.0, for at least 30 min at 60°C. Avoid drying the DNA pellet for more than 10 min at room temperature as over-dried genomic DNA is very difficult to redissolve. Load 3–5  $\mu$ g of DNA per well. Standard PFGE conditions are as follows: 1% agarose gel in 0.5 x TBE electrophoresis buffer; switch intervals = 5–40 seconds; run time = 17 hours; voltage = 170 V.

## Purification of high-molecular-weight DNA

QIAGEN® Genomic-tips are recommended for large-scale purification of high-molecular-weight DNA. QIAGEN Genomic-tips are available for purification of up to 500  $\mu$ g of genomic DNA ranging in size from 50 to 150 kb. They are highly suited for use in Southern blotting, library construction, genome mapping, and other demanding applications. Please contact Technical Services or your local distributor for more information.

# DNeasy Protocol for Animal Tissues

## Important notes before starting

- Before using the DNeasy Tissue Kit for the first time, please read “Technical Information” on pages 9–16.
- Buffers ATL and AL\* may form precipitates upon storage. If a precipitate has formed in either buffer, incubate the Buffer at 55°C until the precipitate has fully dissolved.
- Buffers AW1\* and AW2† are supplied as concentrates. Before using for the first time, add the appropriate amounts of ethanol (96–100%) to Buffers AW1 and AW2 as indicated on the bottles.
- Prepare a 55°C shaking water bath for use in step 2 and a 70°C water bath or heating block for use in step 3.
- If using frozen material, equilibrate the sample to room temperature.
- To isolate DNA from fixed tissue, please refer to “Guidelines for preparation and lysis of other starting material” (starting on page 25).
- All centrifugation steps are carried out at room temperature at  $\geq 6000 \times g$ .
- Vortexing should be performed by pulse vortexing for 5–10 s.
- Optionally, RNase A may be used to digest RNA during the procedure. RNase A is not provided in the DNeasy Tissue Kit (see “Copurification of RNA”, page 12).

### 1. Cut up to 25 mg tissue (up to 10 mg spleen) into small pieces, place in a 1.5 ml microcentrifuge tube, and add 180 $\mu$ l Buffer ATL.

Ensure the correct amount of starting material is used (see page 9). For tissues such as spleen with a very high number of cells for a given mass of tissue, no more than 10 mg starting material should be used.

It is advisable to cut the tissue into small pieces to enable more efficient lysis.

### 2. Add 20 $\mu$ l proteinase K, mix by vortexing, and incubate at 55°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a shaking water bath or on a rocking platform.

Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.

After incubation, the lysate may appear viscous but should not be gelatinous as it may clog the DNeasy spin column. If the lysate appears very gelatinous, please see the “Troubleshooting Guide” on page 30 for recommendations.

\* Contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents containing bleach.

† Contains sodium azide as a preservative. Sodium azide is highly toxic and may react explosively with lead or copper drainpipes. Take appropriate safety measures and wear gloves when handling. Flush drains thoroughly with water after disposing of solutions containing sodium azide and follow your institution's waste-disposal guidelines.

**Optional: RNase treatment of the sample. Add 4  $\mu$ l of RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature.**

Transcriptionally active tissues such as liver and kidney contain high levels of RNA, which will copurify with genomic DNA. If RNA-free genomic DNA is required, carry out this optional step. If residual RNA is not a concern, omit this step and continue with step 3.

- 3. Vortex for 15 s. Add 200  $\mu$ l Buffer AL to the sample, mix thoroughly by vortexing, and incubate at 70°C for 10 min.**

It is essential that the sample and Buffer AL are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.

A white precipitate may form on addition of Buffer AL, which in most cases will dissolve during the incubation at 70°C. The precipitate does not interfere with the DNeasy procedure. Some tissue types (e.g., spleen, lung) may form a gelatinous lysate after addition of Buffer AL. In this case, vigorously shaking or vortexing the preparation before addition of ethanol in step 4 is recommended.

- 4. Add 200  $\mu$ l ethanol (96–100%) to the sample, and mix thoroughly by vortexing.**

It is important that the sample, Buffer AL, and the ethanol are mixed thoroughly to yield a homogeneous solution.

A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the DNeasy spin column.

- 5. Pipet the mixture from step 4 into the DNeasy spin column placed in a 2 ml collection tube (provided). Centrifuge at  $\geq 6000 \times g$  (8000 rpm) for 1 min. Discard flow-through and collection tube.**
- 6. Place the DNeasy spin column in a new 2 ml collection tube (provided), add 500  $\mu$ l Buffer AW1, and centrifuge for 1 min at  $\geq 6000 \times g$  (8000 rpm). Discard flow-through and collection tube.**
- 7. Place the DNeasy spin column in a 2 ml collection tube (provided), add 500  $\mu$ l Buffer AW2, and centrifuge for 3 min at full speed to dry the DNeasy membrane. Discard flow-through and collection tube.**

This centrifugation step ensures that no residual ethanol is carried over during the following elution. Discard flow-through and collection tube.

Following the centrifugation step, remove the DNeasy spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. (If carryover of ethanol occurs, empty the collection tube and reuse it in another centrifugation step for 1 min at full speed.)

8. Place the DNeasy spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200  $\mu$ l Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at  $\geq 6000 \times g$  (8000 rpm) to elute.

Elution with 100  $\mu$ l (instead of 200  $\mu$ l) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield (see Figure 2, page 13).

9. Repeat elution once as described in step 8.

A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 8 can be reused for the second elution step.

**Note:** More than 200  $\mu$ l should not be eluted into a 1.5 ml microcentrifuge tube because the DNeasy mini column will come into contact with the eluate.

# DNeasy Protocol for Rodent Tails

## Important notes before starting

- Before using the DNeasy Tissue Kit for the first time, please read “Technical Information” on pages 9–16.
- Buffers AL\* and ATL may form precipitates upon storage. If a precipitate has formed, incubate the buffer at 55°C until the precipitate has fully dissolved.
- Buffers AW1\* and AW2† are supplied as concentrates. Before using for the first time, add the appropriate amounts of ethanol (96–100%) to Buffers AW1 and AW2 as indicated on the bottles.
- Mix Buffer AL with ethanol for use in step 3. Mix 200 µl Buffer AL and 200 µl ethanol (96–100%) per preparation. The Buffer AL–ethanol mixture is stable for at least 3 months when stored at room temperature (15–25°C).
- Prepare a 55°C shaking water bath for use in step 2.
- If using frozen material, equilibrate the sample to room temperature.
- The law in some countries may dictate the maximum amount of rodent tail that can be removed. This may be less than the amount recommended for use in this protocol.
- All centrifugation steps are carried out at room temperature at  $\geq 6000 \times g$ .
- Vortexing should be performed by pulse vortexing for 5–10 s.
- Optionally, RNase A may be used to digest RNA during the procedure. RNase A is not provided in the DNeasy Tissue Kit (see “Copurification of RNA”, page 12).

### 1. Cut one (rat) or up to two (mouse) 0.4–0.6 cm lengths of tail into a 1.5 ml micro centrifuge tube. Add 180 µl Buffer ATL. Earmark the animal appropriately.

A maximum of 1.2 cm (mouse) or 0.6 cm (rat) tail should be used. When purifying DNA from the tail of an adult mouse or rat, it is recommended to use only 0.4–0.6 cm.

### 2. Add 20 µl proteinase K, mix by vortexing, and incubate at 55°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a shaking water bath or on a rocking platform.

After mixing the tail section with Buffer ATL and proteinase K, ensure the tail section is fully submerged.

Lysis is usually complete in 6–8 h. If it is more convenient, samples can be lysed overnight.

The lysate may appear viscous but should not be gelatinous as it may clog the DNeasy mini column. If the lysate still appears very gelatinous after incubation and vortexing, refer to the “Troubleshooting Guide” on page 30 for recommendations.

\* Contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents that contain bleach.

† Contains sodium azide as a preservative. Sodium azide is highly toxic and may react explosively with lead or copper drainpipes. Take appropriate safety measures and wear gloves when handling. Flush drains thoroughly with water after disposing of solutions containing sodium azide and follow your institution's waste-disposal guidelines.

**Optional: Add 4  $\mu$ l of RNase A (100 mg/ml) to the sample, mix by vortexing, and incubate for 2 min at room temperature.**

Rodent tail tissue contains low levels of RNA, which will be copurified. RNase A digestion can be used to destroy any residual RNA.

If residual RNA is not a concern, continue with step 3.

- 3. Vortex for 15 s. Add 400  $\mu$ l Buffer AL–ethanol mixture (see “Important notes before starting”, page 20) to the sample, and mix vigorously by vortexing.**

It is essential that the sample and Buffer AL–ethanol mixture are mixed thoroughly to yield a homogeneous solution.

A white precipitate may form on addition of the Buffer AL–ethanol mixture. It is essential to pipet all the precipitate into the DNeasy spin column. This precipitate does not interfere with the DNeasy procedure or with any subsequent application.

- 4. Pipet the mixture from step 3 into the DNeasy spin column placed in a new 2 ml collection tube (provided). Centrifuge at  $\geq 6000 \times g$  (8000 rpm) for 1 min. Discard flow-through and collection tube.**
- 5. Place the DNeasy spin column in a new 2 ml collection tube (provided), add 500  $\mu$ l Buffer AW1, and centrifuge for 1 min at  $\geq 6000 \times g$  (8000 rpm). Discard flow-through and collection tube.**
- 6. Place the DNeasy spin column in a new 2 ml collection tube (provided), add 500  $\mu$ l of Buffer AW2, and centrifuge for 3 min at full speed to dry the DNeasy membrane. Discard flow-through and collection tube.**

It is important to dry the membrane of the DNeasy spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution. Discard flow-through and collection tube.

Following the centrifugation step, remove the DNeasy spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. (If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at full speed.)

- 7. Place the DNeasy spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided) and pipet 200  $\mu$ l Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at  $\geq 6000 \times g$  (8000 rpm) to elute.**

Elution with 100  $\mu$ l (instead of 200  $\mu$ l) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield (see Figure 2, page 13).

**8. Repeat elution once as described in step 7.**

A new microcentrifuge tube (not provided) can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 7 can be reused for the second elution step.

**Note:** More than 200  $\mu$ l should not be eluted into a 1.5 ml microcentrifuge tube because the DNeasy spin column will come into contact with the eluate.

# DNeasy Protocol for Cultured Animal Cells

## Important notes before starting

- Before using the DNeasy Tissue Kit for the first time, please read “Technical Information” on pages 9–16.
- Buffer AL\* may form a precipitate upon storage. If a precipitate has formed, incubate the buffer at 55°C until the precipitate has fully dissolved.
- Buffers AW1\* and AW2† are supplied as concentrates. Before using for the first time, add the appropriate amounts of ethanol (96–100%) to Buffers AW1 and AW2 as indicated on the bottles.
- Prepare a 70°C water bath or heating block for use in step 2.
- All centrifugation steps are carried out at room temperature at  $\geq 6000 \times g$ .
- Vortexing should be performed by pulse vortexing for 5–10 s.
- Optionally, RNase A may be used to digest RNA during the procedure. RNase A is not provided in the DNeasy Tissue Kit (see “Copurification of RNA”, page 12).
- Prepare PBS (phosphate-buffered saline) for use in step 1.

### 1. Centrifuge the appropriate number of cells (max. $5 \times 10^6$ ) for 5 min at $300 \times g$ . Resuspend pellet in 200 $\mu$ l PBS (not supplied).

When using a frozen cell pellet, before adding PBS allow cells to thaw until the pellet can be dislodged by gently flicking the tube.

Ensure an appropriate amount of cells is used in the procedure. For cell lines with a high degree of ploidy (e.g., HeLa cells), it is recommended to use less than the maximum number of cells listed in Table 1 (page 9).

**Optional: If RNA-free genomic DNA is required, add 4  $\mu$ l RNase A (100 mg/ml) and incubate for 2 min at room temperature.**

If residual RNA is not a concern, continue with step 2.

### 2. Add 20 $\mu$ l proteinase K and 200 $\mu$ l Buffer AL to the sample, mix thoroughly by vortexing, and incubate at 70°C for 10 min.

It is essential that the sample and Buffer AL are mixed immediately and thoroughly by vortexing and pipetting to yield a homogeneous solution.

**Note:** Do not add proteinase K directly to Buffer AL.

\* Contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents that contain bleach.

† Contains sodium azide as a preservative. Sodium azide is highly toxic and may react explosively with lead or copper drainpipes. Take appropriate safety measures and wear gloves when handling. Flush drains thoroughly with water after disposing of solutions containing sodium azide and follow your institution's waste-disposal guidelines.

**3. Add 200  $\mu$ l ethanol (96–100%) to the sample, and mix thoroughly by vortexing.**

It is important that the sample, Buffer AL, and the ethanol are mixed thoroughly to yield a homogeneous solution.

A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the DNeasy spin column. This precipitate does not interfere with the DNeasy procedure.

**4. Pipet the mixture from step 3 (including any precipitate) into the DNeasy spin column placed in a 2 ml collection tube (provided). Centrifuge at  $\geq 6000 \times g$  (8000 rpm) for 1 min. Discard flow-through and collection tube.**

**5. Place the DNeasy spin column in a new 2 ml collection tube (provided), add 500  $\mu$ l Buffer AW1, and centrifuge for 1 min at  $\geq 6000 \times g$  (8000 rpm). Discard flow-through and collection tube.**

**6. Place the DNeasy spin column in a new 2 ml collection tube (provided), add 500  $\mu$ l Buffer AW2, and centrifuge for 3 min at full speed to dry the DNeasy membrane. Discard flow-through and collection tube.**

It is important to dry the membrane of the DNeasy spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution. Discard flow-through and collection tube.

Following the centrifugation step, remove the DNeasy spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. (If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at full speed.)

**7. Place the DNeasy spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200  $\mu$ l Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at  $\geq 6000 \times g$  (8000 rpm) to elute.**

Elution with 100  $\mu$ l (instead of 200  $\mu$ l) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield (see Figure 2, page 13).

**8. Repeat elution once as described in step 7.**

A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 7 can be reused for the second elution step.

**Note:** More than 200  $\mu$ l should not be eluted into a 1.5 ml microcentrifuge tube because the DNeasy spin column will come into contact with the eluate.

## Guidelines for Preparation and Lysis of Other Starting Materials

In this section, protocols for purification of DNA from additional starting materials are provided. These protocols generally differ from the standard protocols only at the sample-preparation stage.

QIAGEN is continuously developing and optimizing DNeasy protocols for new sample sources. We may already have new protocols of interest to you that are not yet listed here. Please contact Technical Services or your local distributor for more information.

### Protocols for animal blood

The DNeasy procedure has been successfully used for DNA isolation from animal blood (see references, page 34) as well as buffy coat and bone marrow.

Two different protocols are given below for blood samples with non-nucleated (mammalian) and nucleated (e.g., fish, frog, or bird) erythrocytes.

#### **Additional reagent required:**

PBS (phosphate-buffered saline)

50 mM potassium phosphate

150 mM NaCl

pH 7.2

### Isolation of DNA from whole non-nucleated blood

(Tested for use with mouse, rat, guinea pig, hamster, rabbit, cow, and monkey blood.)

1. Pipet 20  $\mu$ l proteinase K into the bottom of a 1.5 ml microcentrifuge tube (not provided).
2. Add 50–100  $\mu$ l anticoagulated blood.
3. Adjust volume to 220  $\mu$ l with PBS.
4. Add 200  $\mu$ l Buffer AL. Mix thoroughly by vortexing.
5. Incubate for 10 min at 70°C.
6. Continue with step 3 of the “DNeasy Protocol for Cultured Animal Cells” (page 24).

## Isolation of genomic DNA from whole nucleated blood

(Tested for use with chicken and goldfish blood.)

1. Pipet 20  $\mu$ l proteinase K into the bottom of a 1.5 ml microcentrifuge tube (not provided).
2. Add 5–10  $\mu$ l anticoagulated blood.
3. Adjust volume to 220  $\mu$ l with PBS.
4. Add 200  $\mu$ l Buffer AL. Mix thoroughly by vortexing.
5. Incubate for 10 min at 70°C.
6. Continue with step 3 of the “DNeasy Protocol for Cultured Animal Cells” (page 24).

## Protocols for fixed tissues

The DNeasy Tissue Kit has been successfully used to isolate DNA from fixed tissues. However, the length of DNA isolated from fixed tissues is usually <650 bp, depending on the type and age of the sample and the quality of the fixative used (Wright, D. and Manos, M. [1990] Sample preparation from paraffin-embedded tissues. In: Innis, M., Gelfont, D., Sninsky, J., White, T., eds. PCR Protocols: A guide to methods and applications. San Diego, Academic Press, 153).

Use of fixatives such as alcohol and formalin is recommended. Fixatives that cause cross-linking, such as osmic acid, are not recommended as it can be difficult to obtain amplifiable DNA from tissue fixed with these agents.

### Additional reagents required:

Xylene (for paraffin-embedded tissues)

Ethanol 96–100% (for paraffin-embedded tissues)

PBS (for formalin-fixed tissues; see page 25)

## Isolation of DNA from paraffin-embedded tissue

This protocol describes the removal of paraffin by extraction with xylene. The tissue sample is then processed according to the DNeasy Protocol for Animal Tissues (page 17).

### Important notes before starting

- Lysis time will vary from sample to sample depending on the type of tissue processed.
  - Yields will depend both on the size and the age of the sample processed. Reduced yields compared to fresh or frozen tissues are to be expected. Therefore, eluting the DNA in 50–100  $\mu$ l Buffer AE is recommended.
1. Place a small section (not more than 25 mg) of paraffin-embedded tissue in a 2 ml microcentrifuge tube (not provided).
  2. Add 1200  $\mu$ l xylene. Vortex vigorously.

3. Centrifuge at full speed for 5 min at room temperature.
4. Remove supernatant by pipetting. Do not remove any of the pellet.
5. Add 1200  $\mu$ l absolute ethanol to the pellet to remove residual xylene and mix gently by vortexing.
6. Centrifuge at full speed for 5 min at room temperature.
7. Carefully remove the ethanol by pipetting. Do not remove any of the pellet.
8. Repeat steps 5–7 once.
9. Incubate the open microcentrifuge tube at 37°C for 10–15 min until the ethanol has evaporated.
10. Resuspend the tissue pellet in 180  $\mu$ l Buffer ATL and continue with the “DNeasy Protocol for Animal Tissues” ( from step 2, page 17).

## Protocol for isolation of DNA from formalin-fixed tissues

### Important notes before starting

- Lysis time will vary from sample to sample depending on the type of tissue processed.
  - Yields will depend both on the size and the age of the sample processed. Reduced yields compared to fresh or frozen tissues are to be expected. Therefore, eluting the DNA in 50–100  $\mu$ l Buffer AE is recommended.
1. Wash tissue sample twice with PBS (see page 25) to remove fixative.
  2. Discard PBS and continue with the “DNeasy Protocol for Animal Tissues” (page 17).

## Protocols for bacteria

These protocols have been used successfully to isolate DNA from bacteria such as *E. coli*, *Corynebacterium* spp., and *B. subtilis*. Some bacteria (particularly Gram-positive bacteria) require prior incubation with specific enzymes such as lysozyme to lyse their substantial cell walls. In these cases, follow Protocol B.

Please see “Quantification of starting material” on page 11 for details of how to collect and store samples, and how to determine the number of cells in a bacterial culture.

### A. Isolation of genomic DNA from Gram-negative bacteria

1. Harvest cells (max.  $2 \times 10^9$  cells) in a microcentrifuge tube by centrifuging for 10 min at 7500 rpm (5000  $\times$  g). Discard supernatant.
2. Resuspend pellet in 180  $\mu$ l Buffer ATL.
3. Continue with the “DNeasy Protocol for Animal Tissues” (from step 2, page 17).

## B. Isolation of genomic DNA from Gram-positive bacteria

### Additional reagents required:

Buffer for enzymatic lysis; add lysozyme just before use

20 mM Tris-Cl, pH 8.0

2 mM EDTA

1.2% Triton® X-100

20 mg/ml lysozyme

1. Harvest cells (max.  $2 \times 10^9$  cells) in a microcentrifuge tube by centrifuging for 10 min at 7500 rpm (5000 x g). Discard supernatant.
2. Resuspend bacterial pellet in 180  $\mu$ l enzymatic lysis buffer.
3. Incubate for at least 30 min at 37°C.
4. Add 25  $\mu$ l proteinase K and 200  $\mu$ l Buffer AL. Mix by vortexing.

**Note:** Do not add proteinase K directly to Buffer AL.

5. Incubate at 70°C for 30 min.

**Optional: If required, incubate at 95°C for 15 min to inactivate pathogens. Note that incubation at 95°C can lead to some DNA degradation.**

6. Continue with the “DNeasy Protocol for Animal Tissues” (from step 4, page 17).

## Protocol for isolation of genomic DNA from yeast

In this protocol, the cell wall of yeast cells is lysed enzymatically with lyticase. Spheroplasts are then collected by centrifugation and processed according to the “DNeasy Protocol for Animal Tissues”, page 17.

Please see “Quantification of starting material” on page 11 for details of how to collect and store samples, and how to determine the number of cells in a yeast culture.

### Additional reagents required:

Sorbitol buffer: 1 M sorbitol

100 mM EDTA

14 mM  $\beta$ -mercaptoethanol

Lyticase (yeast-lysing enzyme)

1. Harvest cells (max.  $5 \times 10^7$ ) by centrifuging for 10 min at 7500 rpm (5000 x g). Discard supernatant.
2. Resuspend the pellet in 600  $\mu$ l sorbitol buffer. Add 200 U lyticase and incubate at 30°C for 30 min.

**Note:** lysis time and yield will vary from sample to sample depending on the cell number and species processed. Please refer to enzyme supplier for further guidelines.

3. Pellet the spheroplasts by centrifuging for 10 min at 300 x g.
4. Resuspend the spheroplasts in 180 µl Buffer ATL.
5. Continue with the “DNeasy Protocol for Animal Tissues” (from step 2, page 17).

## Protocols for isolation of genomic DNA from insects

The DNeasy Tissue Kit has been used to isolate genomic DNA from drosophila.

Use either Protocol A or Protocol B, as desired.

### Protocol A

1. Grind up to 50 mg insects in liquid nitrogen with mortar and pestle, and place powder in a 1.5 ml microcentrifuge tube.
2. Add 180 µl Buffer ATL.
3. Follow the “DNeasy Protocol for Animal Tissues” (from step 2, page 17).

### Protocol B

1. Place up to 50 mg insects in a 1.5 ml microcentrifuge tube.
2. Add 180 µl PBS (see page 25) and homogenize the sample using an electric homogenizer or a disposable microtube pestle.
3. Continue with the “DNeasy Protocol for Cultured Animal Cells” (from step 2, page 23).

## Protocol for isolation of genomic DNA from crude lysates

For preparation of genomic DNA from samples other than those listed in this handbook or for which specialized protocols are not available, the following procedure is recommended.

### Important note before starting

Optimal lysis conditions must first be determined for the chosen sample. DNeasy lysis buffers may not be suitable for all sample sources.

1. Lyse sample in 200 µl of a sample-specific lysis buffer.  
If a larger volume of lysis buffer is required, use 400 µl lysis buffer and double the amounts of proteinase K, Buffer AL, and ethanol in the following steps.
2. Add 20 µl proteinase K (20 mg/ml).
3. Add 200 µl Buffer AL and mix immediately by vortexing.
4. Incubate at 70°C for 10 min.  
Check the pH of the lysate. The lysate must be acidic (pH <7.0) to obtain maximum binding of DNA to the DNeasy membrane.
5. Continue with the “DNeasy Protocol for Animal Tissues” (from step 4, page 18).

# Troubleshooting Guide

Protocols	Observation or possible explanation	Comments and suggestions
<b>Low yield</b>		
<i>All</i>	Storage of starting material	DNA yield is dependent on the type, size, age, and storage of starting material. Lower yields will be obtained from material that has been inappropriately stored (see “Sample collection and storage”, page 9).
<i>All</i>	Too much starting material	In future preparations, reduce the amount of starting material used (see “Quantification of starting material”, page 11).
<i>Animal tissues, rodent tails</i>	Insufficient lysis	<p>In future preparations, extend incubation time at 55°C for proteinase K digestion and/or increase amount of proteinase K to 40 µl.</p> <p>Cut tissue into smaller pieces to facilitate lysis. After lysis, vortex sample vigorously; this will not damage or reduce the size of the DNA.</p> <p>If a substantial gelatinous pellet remains after incubation and vortexing, extend incubation time at 55°C for proteinase K digest and/or increase amount of proteinase K to 40 µl.</p> <p>Ensure the sample is fully submerged in the buffer containing proteinase K. If necessary, double the amount of Buffer ATL and proteinase K and use a 2 ml microcentrifuge tube for lysis. Remember to adjust the amount of Buffer AL and ethanol proportionately in subsequent steps (a lysis step with 360 µl Buffer ATL plus 40 µl proteinase K will require 400 µl Buffer AL plus 400 µl ethanol to bind DNA to the DNeasy membrane). Pipet sample into the DNeasy spin column in two sequential loading steps. Discard flow-through between these loading steps.</p>

<b>Protocols</b>	<b>Observation or possible explanation</b>	<b>Comments and suggestions</b>
<b>Low yield</b>		
<i>Bacteria, yeast</i>	Insufficient lysis	In future preparations, extend incubation time with cell-wall-lysing enzyme and/or increase amount of lysing enzyme.  Harvest bacteria or yeast during the early log phase of growth (see "Sample collection and storage", page 9).
<i>All</i>	Insufficient mixing of sample with Buffer AL and ethanol prior to binding	In future preparations, mix sample with Buffer AL and ethanol by pulse vortexing for 15 s. prior to applying the sample to the DNeasy spin column.
<i>All</i>	DNA inefficiently eluted	Increase elution volume to 200 µl and perform another elution step. See also "Elution of pure nucleic acids", page 13. Check that ethanol was added prior to applying sample to the DNeasy spin column. Check that any precipitate in Buffer ATL and/or Buffer AL was dissolved before use.
<i>All</i>	DNA not bound to DNeasy spin column	Check that ethanol was added prior to applying sample to the DNeasy spin column.
<i>All</i>	Buffer AW1 or AW2 prepared incorrectly	Make sure that ethanol has been added to Buffers AW1 and AW2 before use.
<i>All</i>	Water used instead of Buffer AE for elution	The low pH of deionized water from some water purifiers may reduce DNA yield. When eluting with water, ensure that the pH of the water is at least 7.0.
<b>DNeasy spin column clogged</b>		
<i>All</i>	Too much starting material and/or insufficient lysis	Increase g-force and/or duration of centrifugation step. See also "Too much starting material" and "Insufficient lysis" above.

<b>Protocols</b>	<b>Observation or possible explanation</b>	<b>Comments and suggestions</b>
<b>Discolored membrane after wash with Buffer AW2, or colored eluate</b>		
<i>Rodent tails</i>	Hair not removed during preparation	In future preparations, centrifuge lysate for 5 min at full speed after digestion with proteinase K. Transfer supernatant into a new tube before proceeding with step 3.
<i>Animal blood</i>	Contamination with hemoglobin	Reduce amount of blood used and/or double the amount of proteinase K used per preparation. Try using buffy coat instead of whole blood.
<b>Low concentration of DNA in eluate</b>		
<i>All</i>	Second elution step diluted DNA	Use a new collection tube for the second eluate to prevent dilution of the first eluate. Reduce elution volume to 50–100 $\mu$ l. See “Elution of pure nucleic acids”, page 13.
<b><math>A_{260}/A_{280}</math> ratio of purified DNA is low</b>		
<i>All</i>	Water used instead of buffer to measure $A_{260}$	Use 10 mM Tris-Cl, pH 8.0 instead water to dilute the sample before measuring purity. See “Determination of yield and purity”, page 15.
<i>All</i>	Inefficient cell lysis	See “Low yield” above.
<b><math>A_{260}/A_{280}</math> ratio of purified DNA is high</b>		
<i>All</i>	High level of residual RNA	Use the optional RNase treatment in the protocol.

<b>Protocols</b>	<b>Observation or possible explanation</b>	<b>Comments and suggestions</b>
<b>DNA does not perform well in downstream application</b>		
<i>All</i>	Salt carryover	a) Ensure that Buffer AW2 has been used at room temperature (15–25°C). b) Ensure that Buffers AW1 and AW2 were added in the correct order.
<i>All</i>	Ethanol carryover	Ensure that when washing with Buffer AW2, the column is spun for 3 min at maximum speed to dry the DNeasy membrane. Following the centrifugation step, remove the DNeasy spin column carefully so that the column does not come into contact with the flow-through. If ethanol is visible in the DNeasy spin column (as either drops or a film), discard the flow-through, keep the collection tube, and centrifuge for a further 1 min at full speed.
<i>All</i>	Too much DNA used	For PCR applications, a single-copy gene can typically be detected after 35 PCR cycles with 100 ng DNA template.
<b>DNA sheared</b>		
<i>All</i>	Sample repeatedly frozen and thawed	Avoid repeated freezing and thawing of starting material.
	Sample too old	Old samples often yield only degraded DNA.
<b>White precipitate in Buffer ATL or AL</b>		
<i>All</i>	White precipitate may form at low temperature after prolonged storage	Any precipitate formed when Buffer ATL or Buffer AL is added must be dissolved by incubating the buffer at 55°C until it disappears.

## Selected References

The DNeasy Tissue Kit was formerly known as the QIAamp<sup>®</sup> Tissue Kit.

### Mouse and rat

1. Klenerman, P., Henzgartner, H., and Zinkernagel, R. M. (1997) A non-retroviral RNA virus persists in DNA form. *Nature* **390**, 298.
2. Ohashi, N., Zhi, N., Zhang, Y., and Rikihisa, Y. (1998) Immunodominant major outer membrane proteins of *Ehrlichia chaffeensis* are encoded by a polymorphic multigene family. *Infect. Immun.* **66**, 132.
3. Gerloni, M., Ballou, W.R., Billetta, R., and Zanetti, M. (1997) Immunity to *Plasmodium falciparum* malaria sporozoites by somatic transgene immunization. *Nat. Biotech.* **15**, 876.
4. Courtois-Coutry, N., et al. (1997) A tyrosine-based signal targets H/K-ATPase to a regulated compartment and is required for the cessation of gastric acid secretion. *Cell* **90**, 501.
5. Wildfeuer, A., Schlenk, R., and Friedrich, W. (1996) Detection of *Candida albicans* DNA with a yeast-specific primer system by polymerase chain reaction. *Mycoses* **39**, 341.
6. Soloff, R.S., Wang, T.G., Lybarger, L., Dempsey, D., and Chervenak, R. (1995) Transcription of the TCR-beta locus initiates in adult murine bone marrow. *J. Immunol.* **154**, 3888.
7. Kuzmin, A.I., Finegold, M.J., and Eisensmith, R.C. (1997) Macrophage depletion increases the safety, efficacy and persistence of adenovirus-mediated gene transfer in vivo. *Gene Ther.* **4**, 309.
8. Nevels, M., Rubenwolf, S., Spruss, T., Wolf, H., and Dobner T. (1997) The adenovirus E4orf6 protein can promote E1A/E1B-induced focus formation by interfering with p53 tumor suppressor function. *Proc. Natl. Acad. Sci. USA* **94**, 1206.
9. Xiong, S, Gerloni, M., and Zanetti, M. (1997) In vivo role of B lymphocytes in somatic transgene immunization. *Proc. Natl. Acad. Sci. USA* **94**, 6352.
10. Rapp, J.P., Garrett, M.R., Deng, A.Y. (1998) Construction of a double congenic strain to prove an epistatic interaction on blood pressure between rat chromosome 2 and 10. *J. Clin. Invest.* **101**, 1591.
11. Jamieson, B.D. and Zack, J.A. (1998) In vivo pathogenesis of a human immunodeficiency virus type 1 reporter virus. *J. Virol.* **72**, 6520.

### Xenopus and frog

1. Kroll, K.L. and Amaya, E. (1996) Transgenic xenopus embryos from sperm nuclear transplantations reveal FGF signaling requirements during gastrulation. *Development* **122**, 3173.
2. Fu, Y., Wang, Y., and Evans, S.M. (1998) Viral sequences enable efficient and tissue-specific expression of transgenes in xenopus. *Nat. Biotechnol.* **16**, 253.
3. Berschick, P. (1997) One primer pair amplifies small subunit ribosomal DNA from mitochondria, plastids and bacteria. *Biotechniques* **23**, 494.

## Primates

1. Khan, I.H. (1998) Role of the SH3-ligand domain of simian immunodeficiency virus Nef in interaction with Nef-associated kinase and simian AIDS in Rhesus macaques. *J. Virol.* **72**, 5820.
2. Rey-Cuille, M.A., et al. (1998) Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. *J. Virol.* **72**, 3872.
3. Szkudlinski, M.W., Teh, N.G., Grossmann, M., Tropea, J.E., and Weintraub, B.D. (1996) Engineering human glycoprotein hormone superactive analogues. *Nat. Biotechnol.* **14**, 1257.

## Cells

1. Helps, C.R. and Harbour, D.A. (1997) Comparison of the complete sequences of feline spumavirus with those of the primate spumaviruses reveals a shorter gag gene. *J. Gen. Virol.* **78**, 2549.
2. Rodems, S.M., Clark, C.L., and Spector, D.H. (1998) Separate DNA elements containing ATF/CREB and IE86 binding sites differentially regulate the human cytomegalovirus UL112-113 promoter at early and late times in the infection. *J. Virol.* **72**, 2697.
3. Wei, Q. and Fultz, P.N. (1998) Extensive diversification of human immunodeficiency virus type 1 subtype B strains during dual infection of a chimpanzee that progressed to AIDS. *J. Virol.* **72**, 3005.

## Fish

1. Zheng, W., Strobeck, C., and Stacey, N. (1997) The steroid pheromone 4-pregnen-17 $\alpha$ , 20 $\beta$ -DIOL-3-one increases fertility and paternity in goldfish. *J. Exp. Biology* **200**, 2833.
2. Sultmann, H., Mayer, W.E., Figueroa, F., Tichy, H., and Klein, J. (1995) Phylogenetic analysis of cichlid fishes using nuclear DNA markers. *Mol. Biol. Evol.* **12**, 1033.

## Bacteria

1. Seigner, L. (1997) Der Nachweis von Burkholderia solanacearum mit der PCR – Vergleich verschiedener DNA-Extraktionmethoden. (German with English summary) *Gesunde Pflanzen* **49**, 37.\*
2. Kang, J., Hein, F., Schaffrath, N., and Welters, S. (1996) Rapid isolation of mycobacterial DNA using QIAamp. *QIAGEN News* 1996 No. **1**, 14.

\* The detection of Burkholderia (Pseudomonas) solanacearum using PCR — comparison of different methods for the extraction of DNA.

## Insects

1. Smith, B., and Kelley, M.R. (1994) Rapid genomic DNA purification from *Drosophila melanogaster* for restriction digestion and PCR. *J. NIH Res.* **6**, 78.
2. Kelley, B. (1995) Rapid genomic DNA purification from *Drosophila melanogaster* for restriction and PCR. *QIAGEN News* 1995 No. **1**, 8.
3. Fukatsu, T. And Nikoh, N. (1998) Two intracellular symbiotic bacteria from the mulberry psyllid *Anomonuera mori* (Insecta, Homoptera). *Appl. Environ. Microbiol.* **64**, 3599.
4. Breiner, K. M., Urban, S., and Schaller, H. (1998) Carboxypeptidase D (gp180), a golgi-resident protein, functions in the attachment and entry of avian hepatitis B viruses. *J. Virol.* **72**, 8098

## Other

1. Ge, N.L., et al. (1997) Use of a nonradioactive DNA probe for detection of *Anaplasma marginale* infection in field cattle: comparison with complement fixation serology and microscopic examination. *J. Vet. Diagn. Invest.* **9**, 39.
2. Trontelj, P., Sket, B., Dovc, P., and Steinbrück, G. (1996) Phylogenetic relationships in European erpobdellid leeches (Hirudinea: Erpobdellidae) inferred from restriction-site data of the 18S ribosomal gene and ITS2 region. *G. Zoo. Sys. Evol. Res.* **43**, 85.
3. Shedlock, A.M., Haygood, M.G., Pietsch, T.W., and Bentzen, P. (1997) Enhanced DNA extraction and PCR amplification of mitochondrial genes from formalin-fixed museum specimens. *BioTechniques* **22**, 394.
4. Venables, C., Lysons, R., Horigan, M., Stagg, D., Dawson, M. (1997) Bovine immunodeficiency-like virus: inactivation in milk by pasteurisation. *Vet. Rec.* **140**, 275.
5. Epe, C., Samson-Himmelstjerna, G.V., and Schneider, T. (1997) Differences in ribosomal DNA sequence of lungworm species (Nematoda: Dictyocaulidae) from fallow deer, cattle, sheep and donkeys. *Res. Vet. Sci.* **62**, 17.
6. Storey, J.R., et al. (1998) Molecular cloning and sequencing of three granulocytic ehrlichia genes encoding high-molecular-weight immunoreactive proteins. *Infect. Immun.* **66**, 1356.
7. Berschick, P. (1997) One primer pair amplifies small subunit ribosomal DNA from mitochondria, plastids and bacteria. *BioTechniques* **23**, 494.

## Ordering Information

Product	Contents	Cat. No.
<b>DNeasy Tissue Kits — for DNA isolation from tissues, rodent tails, and cultured cells</b>		
DNeasy Tissue Kit (50)	50 DNeasy Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	69504
DNeasy Tissue Kit (250)	250 DNeasy Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	69506
<b>Related products</b>		
<b>DNeasy 96 Tissue Kits — for high-throughput DNA isolation from rodent tails</b>		
DNeasy 96 Tissue Kit (4)*	For 4 x 96 DNA preps: 4 DNeasy 96 Plates, Proteinase K, Buffers, S-Blocks, AirPore™ Tape Sheets, Collection Microtubes (1.2 ml), Elution Microtubes RS, Caps, 96-well Plate Registers	69581
DNeasy 96 Tissue Kit (12)*	For 12 x 96-well Plate Registers: 12 DNeasy 96 Plates, Proteinase K, Buffers, S-Blocks, AirPore Tape Sheets, Collection Microtubes (1.2 ml), Elution Microtubes RS, Caps, 96-well Plate Registers	69582
<b>Genomic-tips — for small- to large-scale purification of high-molecular-weight DNA</b>		
QIAGEN Genomic-tip 20/G	25 columns	10223
QIAGEN Genomic-tip 100/G	25 columns	10243
QIAGEN Genomic-tip 500/G	10 columns	10262
<b>RNeasy® Kits — for total RNA isolation from animal cells or tissues, yeast, or bacteria</b>		
RNeasy Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104

\* Requires use of the QIAGEN 96-Well-Plate Centrifugation System. For further information, contact QIAGEN Technical Services your local distributor.

## Ordering Information

Product	Contents	Cat. No.
RNeasy Mini Kit (250)	250 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74106
RNeasy Midi Kit (10)	10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-free Reagents and Buffers	75142
RNeasy Midi Kit (50)	50 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-free Reagents and Buffers	75144
RNeasy Maxi Kit (12)	12 RNeasy Maxi Spin Columns, Collection Tubes (50 ml), RNase-free Reagents and Buffers	75162
<b>Accessories</b>		
Buffer AW1 (concentrate, 242 ml)	242 ml Wash Buffer (1) Concentrate for 1000 spin, 250 midi-, or 100 maxipreps	19081
Buffer AW2 (concentrate, 324 ml)	324 ml Wash Buffer (2) Concentrate	19072
Buffer AL (216 ml)	216 ml for 1000 preps	19075
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer for 1000 preps	19076
Buffer AE (240 ml)	240 ml Elution Buffer for 1000 preps	19077
Collection Tubes (2 ml)	1000 Collection Tubes (2 ml)	19201
Genomic DNA Buffer Set*	Buffers, including specific lysis buffers for yeast, bacteria, cells, blood, and tissue: Y1, B1, B2, C1, G2, QBT, QC, QF; for 75 mini-, 25 midi-, or 10 maxipreps	19060
Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
Proteinase K (10 ml)	10 ml (>600 mAU/ml, solution)	19133

\* Enzymes must be purchased separately.

## Trademarks

Patented or patent-pending and/or registered or registration-pending trademarks of the QIAGEN Group: QIAGEN®, QIAamp®, AirPore™, DNeasy®, RNeasy®.

Triton is a registered trademark of Rohm and Haas Company.

Corex is a registered trademark of Corning Glass Works.

TaqMan is a registered trademark of Roche Molecular Systems, Inc.

Purchase of QIAGEN products for PCR is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process for research and development activities in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler. The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Registered names, trademarks, etc., used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

## Notes

**Notes**

## Notes

# QIAGEN Companies



Please see the inside front cover for contact information for your local QIAGEN office.

## QIAGEN Distributors

### Argentina

Tecnolab S.A.  
Charlone 144 - C1427BXD  
Capital Federal  
Tel: (011) 4555 0010  
Fax: (011) 4553 3331  
E-mail: info@tecnolab.com.ar  
Web site: www.tecnolab.com.ar

### Austria/Slovenia

VWR International GmbH  
Zim�abasse 5  
1147 Wien  
Austria  
Tel: (01) 576 00 0  
Fax: (01) 576 00 350  
E-mail: merckwien@merckeurolab.at  
Web site: www.vwr.com

### Belgium/Luxemburg

Westburg b.v.  
P.O. Box 214  
3830 AE Leusden  
The Netherlands  
Tel: 0800-1-9815  
Fax: (31) 33-4951222  
E-mail: info@westburg.nl  
Web site: www.westburg.nl

### Brazil

Uniscience do Brasil  
Av. Candido Portinari, 933/937  
05114-001 Sao Paulo - SP  
Brazil  
Tel: 011 3622 2320  
Fax: 011 3622 2323  
E-mail: info@uniscience.com  
Web site: www.uniscience.com

### China

Gene Company Limited  
Unit A, 8/F., Shell Industrial Building  
12 Lee Chung Street  
Chai Wan, Hong Kong, P.R.C.  
Tel: (852)2896-6283  
Fax: (852)2515-9371  
E-mail:  
Hong Kong: info@genehk.com  
Beijing: gene@public2.bta.net.cn  
Shanghai: gene@public.sta.net.cn  
Chengdu: gene@public.cd.sc.cn  
Guangzhou: gzyitao@public.guangzhou.gd.cn

### Cyprus

Scientronics Ltd  
34, Zenonos Sozou Str.  
1075 Lefkosia  
Tel: 02765 416  
Fax: 02764 614  
E-mail: sarpetso@spidernet.com.cy

### Czech Republic

BIOCONSULT spol. s.r.o.  
Bozejovicka 145  
142 01 Praha-Libuř  
Tel/Fax: (420) 2 417 29 792  
E-mail: bio-cons@login.cz  
Web site: www.bio-consult.cz

### Denmark

VWR International ApS  
Roskildevej 16  
2620 Albertslund  
Tel: 43 86 87 88  
Fax: 43 86 87 90  
E-mail: info@dk.vwr.com  
Web site: www.vwr.com

### Egypt

Clinilab  
P.O. Box 12 El-Manial  
4, 160 St., El-Ehmad Square  
Riham Tower, El-Maadi  
Cairo  
Tel: 52 57 212  
Fax: 52 57 210  
E-mail: Clinilab@link.net

### Finland

VWR International Oy  
Niittyrinne 7  
02270 Espoo  
Tel: (09) 804 551  
Fax: (09) 8045 5200  
E-mail: info@fi.vwr.com  
Web site: www.vwr.com

### Greece

BioAnalytica S.A.  
11, Laskareos Str.  
11471 Athens  
Tel: (10)640 03 18  
Fax: (10)646 27 48  
E-mail: bioanalyt@hol.gr

### India

Genetix  
C-88, Kirti Nagar  
Lower Ground Floor  
New Delhi-110 015  
Tel: (011)-542 1714  
or (011)-515 9346  
Fax: (011)-546 7637  
E-mail: genetix@nda.vsnl.net.in

### Israel

Westburg [Israel] Ltd.  
1, Habursekai St. Kiriat Ha'asakim  
Beer Sheva 84899  
Tel: 08-6650813/4  
or 1-800 20 22 20 (toll free)  
Fax: 08-6650934  
E-mail: info@westburg.co.il  
Web site: www.westburg.co.il

### Korea

LRs Laboratories, Inc.  
Songbuk P.O. Box 61  
Seoul, 136-600  
Tel: (02) 924-86 97  
Fax: (02) 924-86 96  
E-mail: webmaster@lrslab.co.kr  
Web site: www.lrslab.co.kr

### Malaysia

RESEARCH BIOLABS SDN. BHD.  
11-A, Jalan BK 5A/2  
Bandar Kinrara  
47100 Puchong, Selangor Darul Ehsan  
Tel: (603)-8070 3101  
Fax: (603)-8070 5101  
E-mail: biolabs@tm.net.my  
Web site: www.researchbiolabs.com

### Mexico

Quimica Valner S.A. de C.V.  
Jalapa 77, Col Roma  
Mexico D.F. 06700  
Tel: (55) 55 25 57 25  
Fax: (55) 55 25 56 25  
E-mail: qvalaner@infosel.net.mx

### The Netherlands

Westburg b.v.  
P.O. Box 214  
3830 AE Leusden  
Tel: (033)4950094  
Fax: (033)4951222  
E-mail: info@westburg.nl  
Web site: www.westburg.nl

### New Zealand

Biolab Scientific Ltd.  
244 Bush Road  
Albany, Auckland  
Tel: (09)9806700  
or 0800933966  
Fax: (09)9806788  
E-mail: info@biolab.co.nz  
Web site: www.biolab.co.nz

### Norway

VWR International AS  
Kakkelovnskroken 1  
P.B. 45, Kalbakken,  
0901 Oslo  
Tel: 22 90 00 00  
Fax: 22 90 00 40  
E-mail: info@no.vwr.com  
Web site: www.vwr.com

### Poland

Syngen Biotech Sp.z.o.o.  
ul. Legnicka 62 A  
54-204 Wrocław  
Tel: (071) 351 41 06  
or 0601 70 60 07  
Fax: (071) 351 04 88  
E-mail: info@syngen.com.pl  
Web site: www.syngen.com.pl

### Portugal

IZASA PORTUGAL, LDA  
Rua do Proletariado, 1 - Quinta do  
Paizinho  
2795-648 Carnaxide  
Tel: (21) 424 7312  
Fax: (21) 417 2674

### Singapore

Research Biolabs Pte Ltd  
211 Henderson Road #14-01  
Henderson Industrial Estate  
Singapore 159552  
Tel: 2731066  
Fax: 2734914  
E-mail: biolabs@singnet.com.sg

### Slovak Republic

BIOCONSULT Slovakia spol. s.r.o.  
Ružova dolina 6  
SK-821 08 Bratislava 2  
Tel/Fax: (02) 5022 1336  
E-mail: bio-cons@post.sk  
Web site: www.bio-consult.cz

### South Africa

Southern Cross Biotechnology (Pty) Ltd  
P.O. Box 23681  
Claremont 7735  
Cape Town  
Tel: (021) 671 5166  
Fax: (021) 671 7734  
E-mail: info@scb.co.za  
Web site: www.scb.co.za

### Spain

IZASA, S.A.  
Aragon, 90  
08015 Barcelona  
Tel: (93) 902.20.30.90  
Fax: (93) 902.22.33.66  
E-mail: suministros@izasa.es

### Sweden

VWR International AB  
Fagerstagatan 18A  
163 94 Stockholm  
Tel: (08) 621 34 00  
Fax: (08) 760 45 20  
E-mail: info@se.vwr.com  
Web site: www.vwr.com

### Taiwan

TAIGEN Bioscience Corporation  
3F, No. 306, Section 4  
Chen-Der Road  
111 Taipei  
Taiwan, R.O.C.  
Tel: (02) 2880 2913  
Fax: (02) 2880 2916  
E-mail: taigen@ms10.hinet.net

### Thailand

Theera Trading Co. Ltd.  
64 Charan Sanit Wong Road  
(Charan 13) Bangkokyai  
Bangkok 10600  
Tel: (02) 412-5672  
Fax: (02) 412-3244  
E-mail: theetrad@samart.co.th

### QIAGEN Importers

#### Central & South America

(except Argentina & Brazil)  
Labtrade Inc.  
6157 NW 167th Street F-26  
Miami, FL 33015  
USA  
Tel: (305) 828-3818  
Fax: (305) 828-3819  
E-mail: labtrade@iccnec.net  
Web site: www.labtrade.com

#### Hungary

Kasztel-Med Co. Ltd.  
Kelenhegyi út 22  
H-1118  
Tel: (01) 385 3887  
Fax: (01) 3810695  
E-mail: info@kasztel.hu  
Web site: kasztel.hu

#### Estonia

PEAI-Est OÜ  
Riia 185  
51014 Tartu  
Tel: (051) 65 830  
Fax: (07) 383 360  
E-mail: langel@ut.ee

#### Saudi Arabia

Abdulla Fouad Co. Ltd.  
Medical Supplies Division  
Prince Mohammed Street  
P.O. Box 257, Dammam 31411  
Kingdom of Saudi Arabia  
Tel: (03) 8324400  
Fax: (03) 8346174  
E-mail:  
sadiq.omar@abdullo-fouad.com

#### Turkey

Medek Medikal Ürünler  
ve Sağlık Hizmetleri A.Ş.  
Bağdat Cad. 449 D.9 Sudaıye  
81070 İstanbul  
Tel: (216) 302 15 80  
Fax: (216) 302 15 88  
E-mail: akalp@turk.net

#### All other countries

QIAGEN GmbH, Germany

10200930 0.5/2002

