

July 2003

# DNeasy<sup>®</sup> Tissue Handbook

For DNA purification from

animal tissues

rodent tails

cultured cells

bacteria

yeast

animal blood

insects

fixed tissues



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## Kit Contents

<b>DNeasy® Tissue Kit</b>		
<b>Catalog no.</b>	<b>69504</b>	<b>69506</b>
<b>Number of preps</b>	<b>50</b>	<b>250</b>
DNeasy Mini Spin 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 <sup>†</sup> (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. Not compatible with disinfecting agents containing bleach. For more information, see page 7.

<sup>†</sup> Contains sodium azide as a preservative. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

## Storage

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 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.

## 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 back 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 DNeasy Tissue Kits or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

## Quality Control

As part of the stringent QIAGEN quality assurance program, the performance of DNeasy Tissue Kits is monitored routinely on a lot-to-lot basis. All components are tested separately to ensure highest performance and reliability.

## 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 spilt, clean with suitable laboratory detergent and water. If the spilt 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)**

Contain 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**

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

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

\* 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 breathe 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.

## 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 Spin 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

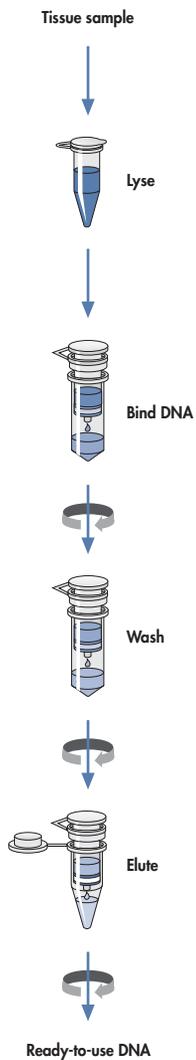
For high-throughput needs, QIAGEN offers the DNeasy 96 Tissue Kit — DNeasy technology in a convenient, 96-well format for DNA isolation from rodent tails and other tissues. Please contact QIAGEN Technical Services or your local distributor for more information.

## Equipment and Reagents to Be Supplied by User

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

- 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%)

# DNeasy Tissue Procedure



## Important Notes

### 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.

Following proteinase K digestion, tissue samples can also be stored in Buffer ATL 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

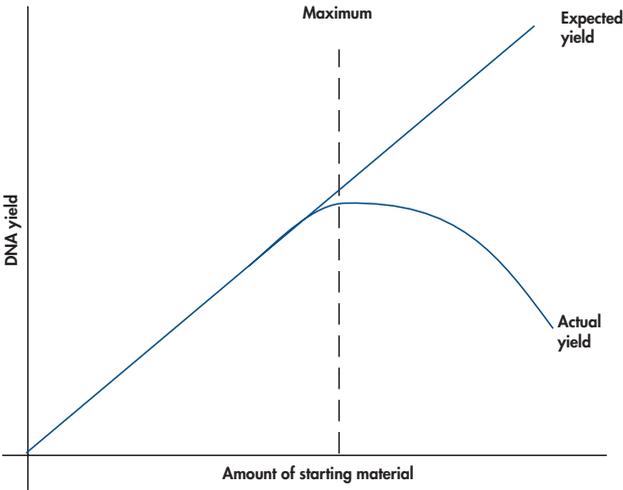
In order to obtain optimum DNA yield and quality, it is important not to overload the DNeasy procedure, as this can lead to significantly lower yields than expected (see Figure 1, next page). For samples with very high DNA contents (e.g., spleen, which has a high cell density, and cell lines with a high 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 15) 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 to 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  $1\text{--}2 \times 10^7$  cells per ml.

## Proteinase K

DNeasy Tissue Kits contain ready-to-use proteinase K supplied in a specially formulated storage buffer. The activity of proteinase K is 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 minutes 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 QIAGEN Technical Services or your local distributor if you have any questions about the use of proteinase K.

## Copurification of RNA

DNeasy Mini Spin Columns copurify DNA and RNA when both are present in the sample (see Table 3, page 15). 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 before 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 Spin 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).

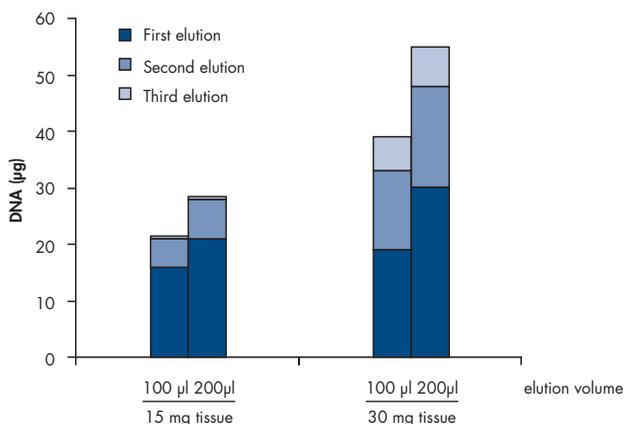
For samples containing up to 10  $\mu$ g DNA, a single elution step using 200  $\mu$ l is sufficient. For samples containing more than 10  $\mu$ g, a second elution step with another 200  $\mu$ l Buffer AE is recommended. Approximately 60–80% of the DNA will elute in the first elution. If >30  $\mu$ 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 DNA per 10<sup>6</sup> cells)

For more details, see Table 3.

**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 0.1 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 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room temperature (15–25°C), 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 0.5x 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 QIAGEN Technical Services or your local distributor for more information.

# Protocol: Isolation of Total DNA from Animal Tissues

## Important points before starting

- Before using the DNeasy Tissue Kit for the first time, please read “Important Notes” on pages 10–17.
- If using fixed tissue, please refer to Appendices B and C (starting on page 31).
- All centrifugation steps are carried out at room temperature (15–25°C).
- 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 13).

## Things to do before starting

- 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<sup>†</sup> 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.

## Procedure

- 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 µl Buffer ATL.**

Ensure the correct amount of starting material is used (see page 10). 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.

\* Contains chaotropic salt. Not compatible with disinfecting agents containing bleach. For more information, see page 7.

<sup>†</sup> Contains sodium azide as a preservative. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, see page 7.

- 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 Mini Spin Column. If the lysate appears very gelatinous, please see the “Troubleshooting Guide” on page 26 for recommendations.

**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 (15–20°C).**

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 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 Mini Spin Column.

- 5. Pipet the mixture from step 4 into the DNeasy Mini 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 Mini 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 Mini Spin Column in a 2 ml collection tube (provided), add 500  $\mu$ l Buffer AW2, and centrifuge for 3 min at 20,000  $\times$  g (14,000 rpm) 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.

Following the centrifugation step, remove the DNeasy Mini 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 20,000  $\times$  g [14,000 rpm].)

8. **Place the DNeasy Mini 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 14).

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 Spin Column will come into contact with the eluate.

# Protocol: Isolation of Total DNA from Rodent Tails

## Important points before starting

- Before using the DNeasy Tissue Kit for the first time, please read “Important Notes” on pages 10–17.
- 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 (15–25°C).
- 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 13).

## Things to do before starting

- 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<sup>†</sup> 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.

## Procedure

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.

\* Contains chaotropic salt. Not compatible with disinfecting agents that contain bleach. For more information, see page 7.

<sup>†</sup> Contains sodium azide as a preservative. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

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.**

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 Spin Column. If the lysate still appears very gelatinous after incubation and vortexing, refer to the “Troubleshooting Guide” on page 26 for recommendations.

**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 (15–25°C).**

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 “Things to do before starting”, page 21) 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 Mini 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 Mini 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 Mini 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 Mini Spin Column in a new 2 ml collection tube (provided), add 500  $\mu$ l of Buffer AW2, and centrifuge for 3 min at 20,000  $\times$  g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.**

It is important to dry the membrane of the DNeasy Mini 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.

Following the centrifugation step, remove the DNeasy Mini 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 20,000  $\times$  g [14,000 rpm].)

7. **Place the DNeasy Mini 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 14).

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 Mini Spin Column will come into contact with the eluate.

# Protocol: Isolation of Total DNA from Cultured Animal Cells

## Important points before starting

- Before using the DNeasy Tissue Kit for the first time, please read “Important Notes” on pages 10–17.
- All centrifugation steps are carried out at room temperature (15–25°C).
- 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 13).

## Things to do before starting

- 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.
- Prepare PBS (phosphate-buffered saline) for use in step 1.

## Procedure

1. **Centrifuge the appropriate number of cells (max.  $5 \times 10^6$ ) for 5 min at 300 x 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 number 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 10).

**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. Not compatible with disinfecting agents that contain bleach. For more information, see page 7.

† Contains sodium azide as a preservative. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

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

It is important that the sample 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 Mini Spin Column. This precipitate does not interfere with the DNeasy procedure.

**4. Pipet the mixture from step 3 (including any precipitate) into the DNeasy Mini 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 Mini 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 Mini Spin Column in a new 2 ml collection tube (provided), add 500  $\mu$ l Buffer AW2, and centrifuge for 3 min at  $20,000 \times g$  (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.**

It is important to dry the membrane of the DNeasy Mini 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.

Following the centrifugation step, remove the DNeasy Mini 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  $20,000 \times g$  [14,000 rpm].)

**7. Place the DNeasy Mini 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 14).

**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 Mini Spin Column will come into contact with the eluate.

# Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol(s) in this handbook or molecular biology applications (see inside front cover for contact information).

This troubleshooting guide is divided into four sections: The first details general troubleshooting relevant to all protocols described in this handbook, the others are specifically for animal tissues and rodent tails, bacteria and yeast, and animal blood respectively.

## All Protocols

### Comments and suggestions

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#### Low yield

- |  |   |
|--|---|
| a) Storage of starting material  | DNA yield is dependent on the type, size, age, and storage of starting material.<br><br>Lower yields will be obtained from material that has been inappropriately stored (see "Sample collection and storage", page 10).  |
| b) Too much starting material  | In future preparations, reduce the amount of starting material used (see "Quantification of starting material", page 12).   |
| c) Insufficient mixing of sample with Buffer AL and ethanol before binding | In future preparations, mix sample with Buffer AL and ethanol by pulse vortexing for 15 s before applying the sample to the DNeasy Mini Spin Column.  |
| d) DNA inefficiently eluted  | Increase elution volume to 200 $\mu$ l and perform another elution step. See also "Elution of pure nucleic acids", page 14. Check that ethanol was added before applying the sample to the DNeasy Mini Spin Column. Check that any precipitate in Buffer ATL and/or Buffer AL was dissolved before use. |
| e) DNA not bound to DNeasy Mini Spin Column                                | Check that ethanol was added before applying the sample to the DNeasy Mini Spin Column.   |
| f) Buffer AW1 or AW2 prepared incorrectly                                  | Make sure that ethanol has been added to Buffers AW1 and AW2 before use.  |

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## Comments and suggestions

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- g) 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.

### DNeasy Mini Spin Column clogged

- Too much starting material and/or insufficient lysis      Increase *g*-force and/or duration of centrifugation step. In future preparations, reduce the amount of starting material used (see "Quantification of starting material", page 12). See also "Insufficient lysis" in the animal tissues and rodent tails section of this troubleshooting guide.

### Low concentration of DNA in the eluate

- Second elution step diluted the 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 14.

### $A_{260}/A_{280}$ ratio of purified DNA is low

- a) Water used instead of buffer to measure  $A_{260}$       Use 10 mM Tris-Cl, pH 8.0 instead of water to dilute the sample before measuring purity. See "Determination of yield and purity", page 16.
- b) Inefficient cell lysis      See "Low yield", above.

### $A_{260}/A_{280}$ ratio of purified DNA is high

- High level of residual RNA      Perform the optional RNase treatment in the protocol.

### DNA does not perform well in downstream applications

- a) Salt carryover      Ensure that Buffer AW2 has been used at room temperature (15–25°C).  
Ensure that Buffers AW1 and AW2 were added in the correct order.
- b) Ethanol carryover      Ensure that when washing with Buffer AW2, the column is centrifuged for 3 min at 20,000  $\times g$  (14,000 rpm) to dry the DNeasy membrane. Following the centrifugation step, remove the DNeasy Mini Spin Column carefully so that the column does not come into contact with the flow-through. If ethanol is visible in the DNeasy Mini Spin Column (as either drops or a film), discard the flow-through, keep the collection tube, and centrifuge for a further 1 min at 20,000  $\times g$ .

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## Comments and suggestions

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- c) Too much DNA used      For PCR applications, a single-copy gene can typically be detected after 35 PCR cycles with 100 ng template DNA.

### DNA sheared

- a) Sample repeatedly frozen and thawed      Avoid repeated freezing and thawing of starting material.
- b) Sample too old      Old samples often yield only degraded DNA.

### White precipitate in Buffer ATL or Buffer AL

White precipitate may form at low temperature after prolonged storage      Any precipitate formed when Buffer ATL or Buffer AL are added must be dissolved by incubating the buffer at 55°C until it disappears.

## Animal Tissue and Rodent Tail Protocols

### Low yield

Insufficient lysis

In future preparations, reduce the amount of starting material used (see "Quantification of starting material", page 12).

Cut tissue into smaller pieces to facilitate lysis. After lysis, vortex sample vigorously; this will not damage or reduce the size of the DNA.

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.

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 Mini Spin Column in two sequential loading steps. Discard flow-through between these loading steps.

### **Discolored membrane after wash with Buffer AW2, or colored eluate**

Hair not removed from rodent tails during preparation      In future preparations, centrifuge lysate for 5 min at 20,000 x g after digestion with proteinase K. Transfer supernatant into a new tube before proceeding with step 3.

## **Bacteria and Yeast Protocols**

### **Low yield**

Insufficient lysis      In future preparations, extend incubation with cell-wall-lysing enzyme and/or increase amount of lysing enzyme.

Harvest bacteria or yeast during early log phase of growth (see "Sample collection and storage", page 10).

## **Animal Blood Protocol**

### **Discolored membrane after wash with Buffer AW2, or colored eluate**

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.

## Appendix A: Isolation of Genomic DNA from Whole Nucleated or Non-Nucleated Animal Blood

The DNeasy procedure has been successfully used for isolation of DNA from blood (see references, page 37) as well as buffy coat and bone marrow. The protocol is suitable for blood samples with non-nucleated\* and nucleated† erythrocytes.

### Additional reagents required

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), available from the product supplier.

- PBS (phosphate-buffered saline)
- 50 mM potassium phosphate
- 150 mM NaCl
- A pH of 7.2 should always be maintained

### Procedure

- A1. Pipet 20  $\mu$ l proteinase K into the bottom of a 1.5 ml microcentrifuge tube (not provided).
- A2. If using non-nucleated blood, add 50–100  $\mu$ l anticoagulated blood. If using nucleated blood, add 5–10  $\mu$ l anticoagulated blood.
- A3. Adjust volume to 220  $\mu$ l with PBS.
- A4. Add 200  $\mu$ l Buffer AL. Mix thoroughly by vortexing.
- A5. Incubate for 10 min at 70°C.
- A6. Continue with step 3 of “Protocol: Isolation of Total DNA from Cultured Animal Cells” on page 25.

## Appendix B: Isolation of Genomic DNA from Paraffin-Embedded Tissue

The DNeasy procedure 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.

\* E.g., mammalian blood; protocol tested using mouse, rat, guinea pig, hamster, rabbit, cow, and monkey blood.

† E.g., fish or frog blood; protocol tested using chicken and goldfish blood.

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.

This protocol describes the removal of paraffin by extraction with xylene. The tissue sample is then processed according to Protocol: Isolation of Total DNA from Animal Tissues (page 18).

### **Additional reagents required**

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), available from the product supplier.

- Xylene
- Ethanol (96–100%)

### **Important points 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.

### **Procedure**

- B1. Place a small section (not more than 25 mg) of paraffin-embedded tissue in a 2 ml microcentrifuge tube (not provided).**
- B2. Add 1200  $\mu$ l xylene. Vortex vigorously.**
- B3. Centrifuge at full speed for 5 min at room temperature (15–25°C).**
- B4. Remove supernatant by pipetting. Do not remove any of the pellet.**
- B5. Add 1200  $\mu$ l absolute ethanol to the pellet to remove residual xylene, and mix gently by vortexing.**
- B6. Centrifuge at full speed for 5 min at room temperature (15–25°C).**
- B7. Carefully remove the ethanol by pipetting. Do not remove any of the pellet.**
- B8. Repeat steps B5–B7 once.**
- B9. Incubate the open microcentrifuge tube at 37°C for 10–15 min until the ethanol has evaporated.**
- B10. Resuspend the tissue pellet in 180  $\mu$ l Buffer ATL and continue with the “Protocol: Isolation of Total DNA from Animal Tissues” (from step 2, page 19).**

## Appendix C: Isolation of Genomic DNA from Formalin-Fixed Tissue

The DNeasy procedure 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.

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 reagent required

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), available from the product supplier.

- PBS

### Important points 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.

### Procedure

- C1. Wash tissue sample twice with PBS to remove fixative.**
- C2. Discard PBS and continue with the “Protocol: Isolation of Total DNA from Animal Tissues” (page 18).**

## Appendix D: Isolation of Genomic DNA from Gram-Negative Bacteria

The DNeasy procedure has been successfully used to isolate DNA from Gram-negative bacteria such as *E. coli*. Please see “Quantification of starting material” on page 12 for details of how to collect and store samples, and how to determine the number of cells in a bacterial culture.

### Procedure

- D1. Harvest cells (max.  $2 \times 10^9$  cells) in a microcentrifuge tube by centrifuging for 10 min at 5000  $\times$  g (7500 rpm). Discard supernatant.**

D2. Resuspend pellet in 180  $\mu$ l Buffer ATL.

D3. Continue with the "Protocol: Isolation of Total DNA from Animal Tissues" (from step 2, page 19).

## Appendix E: Isolation of Genomic DNA from Gram-Positive Bacteria

The DNeasy procedure has been successfully used to isolate DNA from Gram-positive bacteria such as *Corynebacterium* spp. and *B. subtilis*. Some bacteria require prior incubation with specific enzymes such as lysozyme to lyse their substantial cell walls. Please see "Quantification of starting material" on page 12 for details of how to collect and store samples, and how to determine the number of cells in a bacterial culture.

### Additional reagents required

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), available from the product supplier.

- 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

### Procedure

E1. Harvest cells (max.  $2 \times 10^9$  cells) in a microcentrifuge tube by centrifuging for 10 min at 5000 x g (7500 rpm). Discard supernatant.

E2. Resuspend bacterial pellet in 180  $\mu$ l enzymatic lysis buffer.

E3. Incubate for at least 30 min at 37°C.

E4. 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.

E5. 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.

E6. Continue with the "Protocol: Isolation of Total DNA from Animal Tissues" (from step 4, page 19).

## Appendix F: Isolation of Genomic DNA from Yeast

The DNeasy procedure has been successfully used to isolate 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 "Protocol: Isolation of Total DNA from Animal Tissues", page 18. Please see "Quantification of starting material" on page 12 for details of how to collect and store samples, and how to determine the number of cells in a yeast culture.

### Additional reagents required

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), available from the product supplier.

- Sorbitol buffer: 1 M sorbitol
- 100 mM EDTA
- 14 mM  $\beta$ -mercaptoethanol
- Lyticase (yeast-lysing enzyme)

### Procedure

- F1. Harvest cells (max.  $5 \times 10^7$ ) by centrifuging for 10 min at  $5000 \times g$  (7500 rpm). Discard supernatant.
- F2. Resuspend the pellet in 600  $\mu$ l sorbitol buffer. Add 200 units lyticase and incubate at  $30^\circ\text{C}$  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.
- F3. Pellet the spheroplasts by centrifuging for 10 min at  $300 \times g$ .
- F4. Resuspend the spheroplasts in 180  $\mu$ l Buffer ATL.
- F5. Continue with the "Protocol: Isolation of Total DNA from Animal Tissues" (from step 2, page 19).

# Appendix G: Isolation of Genomic DNA from Insects

The DNeasy procedure has been successfully used to isolate genomic DNA from *Drosophila*. If using a mortar and pestle for grinding, follow steps G1–G3. If using an electric homogenizer or disposable microtube pestle, follow steps G4–G6 below. Do not use a mixture of the two protocols.

## Additional reagents required

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), available from the product supplier.

- PBS (if using an electric homogenizer or disposable microtubes pestle, steps G4–G6)

## Procedure

- G1. Grind up to 50 mg insects in liquid nitrogen with mortar and pestle, and place powder in a 1.5 ml microcentrifuge tube.**
  - G2. Add 180  $\mu$ l Buffer ATL.**
  - G3. Follow the “Protocol: Isolation of Total DNA from Animal Tissues” (from step 2, page 19).**
- Or
- G4. Place up to 50 mg insects in a 1.5 ml microcentrifuge tube.**
  - G5. Add 180  $\mu$ l PBS and homogenize the sample using an electric homogenizer or a disposable microtube pestle.**
  - G6. Continue with the “Protocol: Isolation of Total DNA from Cultured Animal Cells” (from step 2, page 24).**

## Appendix H: 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 point before starting

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

### Procedure

#### H1. Lyse sample in 200 $\mu$ l of a sample-specific lysis buffer.

If a larger volume of lysis buffer is required, use 400  $\mu$ l lysis buffer and double the amounts of proteinase K, Buffer AL, and ethanol in the following steps.

#### H2. Add 20 $\mu$ l proteinase K (20 mg/ml).

#### H3. Add 200 $\mu$ l Buffer AL and mix immediately by vortexing.

#### H4. 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.

#### H5. Continue with the "Protocol: Isolation of Total DNA from Animal Tissues" (from step 4, page 19).

# References

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

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

The DNeasy Tissue Kit was formerly known as the QIAamp® Tissue Kit.

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