User-Developed Protocol:

Purification of total DNA from viscous samples using the DNeasy® Blood & Tissue Kit with acetyl cysteine (NALC) pretreatment

This procedure has been adapted by customers from the DNeasy tissue protocol and is for purification of DNA from viscous samples using the DNeasy Blood & Tissue Kit with acetyl cysteine (NALC) pretreatment. **It has not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections and the detailed protocol "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)" in the *DNeasy Blood & Tissue Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier. DNeasy Blood & Tissue Kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by user

- DNeasy Blood & Tissue Kit (cat. no. 69504 or 69506)
- Pipets and pipet tips
- Vortexer
- Microcentrifuge tubes (1.5 ml or 2 ml)
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Thermomixer, shaking water bath, or rocking platform for heating at 56°C
- Ethanol (96–100%)*
- PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl) or centrifugal microconcentrators
- NALC solution:
  - 50 ml 2.94% sodium citrate
  - 50 ml 4% sodium hydroxide
  - 500 mg N-acetyl-L-cysteine (Sigma, cat. no. A7250)†

Important points before starting

- If using the DNeasy Blood & Tissue Kit for the first time, read "Important Notes" in the *DNeasy Blood & Tissue Handbook*.
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Things to do before starting

- Prepare a fresh NALC solution as described on page 1.
- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a thermomixer, shaking water bath, or rocking platform to 56°C for use in step 4.
- If using frozen samples, equilibrate to room temperature. Avoid repeated thawing and freezing of samples since this will lead to reduced DNA size.

Procedure

1. Add 1 volume of NALC solution to 1 volume of sample. Incubate at room temperature (15–25°C) while agitating gently until the sample has attained the desired fluidity.

2. For processing by centrifugation, follow step 2a; for processing with microconcentrators, follow step 2b.

2a. Centrifugation: Pellet the sample by centrifugation and wash with PBS.

2b. Microconcentrators: Concentrate the samples to a volume of ≤100 µl using a centrifugal microconcentrator.

3. Place the sample in a 1.5 ml microcentrifuge tube. Add 180 µl Buffer ATL.

Ensure that the correct amount of starting material is used (see "Starting amounts of samples" in the *DNeasy Blood & Tissue Handbook*).

4. Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.

Lysis time varies depending on the type of sample 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, see the "Troubleshooting Guide" in the *DNeasy Blood & Tissue Handbook* for recommendations.

5. Vortex for 15 s. Add 200µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix again thoroughly by vortexing.

It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the DNeasy procedure. Some sample types may form a gelatinous lysate after addition of Buffer AL and ethanol. In this case, vigorously shaking or vortexing the preparation is recommended.

6. **Pipet the mixture from step 5 (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at ≥6000 x *g* (8000 rpm) for 1 min. Discard flow-through and collection tube.***

7. **Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at ≥6000 x *g* (8000 rpm). Discard flow-through and collection tube.***

8. **Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x *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 x *g* (14,000 rpm).

9. **Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥6000 x *g* (8000 rpm) to elute.**

   Elution with 100 µl (instead of 200 µl) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield (see *DNeasy Blood & Tissue Handbook*).

10. **Recommended: For maximum DNA yield, repeat elution once as described in step 9.**

    This step leads to increased overall DNA yield.

    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 9 can be reused for the second elution step.

    Note: Do not elute more than 200 µl into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate.

Troubleshooting

For general troubleshooting, please consult the Troubleshooting Guide in the *DNeasy Blood & Tissue Handbook*.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.
Selected handbooks can be downloaded from **www.qiagen.com/literature/default.aspx** .

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from **www.qiagen.com/ts/msds.asp**.