

User-Developed Protocol:

Purification of total DNA from soft tissues using the TissueLyser and the DNeasy[®] Blood & Tissue Kit

This procedure has been adapted by customers from the DNeasy tissue protocol and is for purification of DNA from soft tissues using the QIAGEN[®] TissueLyser and the DNeasy Blood & Tissue Kit. **It has not been thoroughly tested and optimized by QIAGEN.**

The TissueLyser allows high-throughput, rapid and effective disruption of up to 48 tissue samples.

Note: This protocol has only been tested with “soft” tissues (e.g., liver, spleen, thymus, heart, kidney, and brain) and may not work with “hard” tissues (e.g., bone, teeth, and skin).

The following guidelines can be used for both fresh and frozen tissues, and for tissues stabilized with RNA^{later}[®] RNA Stabilization Reagent.

IMPORTANT: Please read the “Safety Information” and “Important Notes” sections in the *DNeasy Blood & Tissue Handbook* before beginning this procedure. Ensure that you are familiar with operating the TissueLyser. See the *TissueLyser Handbook*. 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 and the TissueLyser 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)
- TissueLyser (cat. no. 85210 [USA and Canada], 85200 [Japan], or 85220 [rest of world])
- TissueLyser Adapter Set 2 x 24 (cat. no. 69982)
- Stainless Steel Beads, 5 mm (cat. no. 69989)
- 2 ml Safe-Lock microtubes (Eppendorf, cat. no. 0030 120.094)*
- 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%)[†]

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

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

Important points before starting

- If using the DNeasy Blood & Tissue Kit for the first time, read “Important Notes” in the *DNeasy Blood & Tissue Handbook*. Ensure that you are familiar with operating the TissueLyser. See the *TissueLyser 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.
- Use 10 mg tissue as starting material, increasing the amount if the protocol works satisfactorily.

Things to do before starting

- 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 3.

Procedure

- 1. Pipet 180 µl Buffer ATL into a 2 ml Safe-Lock microtube.**
- 2. Add one stainless steel bead to each tube. For best results, use 5 mm (mean diameter) stainless steel beads.**
- 3. Add 10 mg tissue to the tube, and assemble the TissueLyser.**
Use 10 mg tissue as starting material, increasing the amount if the protocol works satisfactorily.
- 4. Homogenize on the TissueLyser for 20 s at 15 Hz. Do not exceed this time as it may result in DNA shearing.**
- 5. Centrifuge the sample briefly to ensure that all the tissue debris is on the bottom of the tube.**
- 6. Add 20 µl proteinase K to the tube.**
Note: Add 40 µl proteinase K if using RNA*later* stabilized tissues.
- 7. Incubate for 56°C for 1 h in a shaker incubator.**
Lysis time varies depending on the sample processed. In general, samples are lysed in 1 h. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.
- 8. Centrifuge the 2 ml microtube briefly to remove drops from inside the lid.**
Optional: If RNA-free genomic DNA is required, add 4 µl RNase (100 mg/ml) and incubate for 5 min at room temperature before continuing with step 9.

- 9. Add 200 µl Buffer AL to the sample (220 µl Buffer AL if using RNA/later stabilized tissues), 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.

- 10. Pipet the mixture from step 9 (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.***
- 11. 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 $\geq 6000 \times g$ (8000 rpm). Discard flow-through and collection tube.***
- 12. 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 $20,000 \times g$ (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).

- 13. 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 $\geq 6000 \times g$ (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*).

- 14. Recommended: For maximum DNA yield, repeat elution once as described in step 13.**
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 13 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.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See *DNeasy Blood & Tissue Handbook* for safety information.

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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.

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