
# User-Developed Protocol:

# Purification of total DNA from compact animal bone using the DNeasy® Blood & Tissue Kit

This procedure has been adapted by customers from the DNeasy tissue protocol and is for purification of DNA from compact bone using the DNeasy Blood & Tissue Kit. **It has not been thoroughly tested and optimized by QIAGEN.**

**IMPORTANT**: Please read the "Safety Information" and "Important Notes" sections 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
- Razor blades and/or sandpaper
- Metal blender (e.g., Waring)*
- Liquid nitrogen
- Ethanol (96–100%)†
- Additional proteinase K (cat. no. 19131 or 19133), Buffer ATL (cat. no. 19076), and Buffer AL (cat. no. 19075) may be needed if the DNeasy Blood & Tissue Kit is used mainly for bone samples

* 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.


**Additional equipment and reagents for >100 mg samples**

- Centrifuge with 50 ml polypropylene centrifuge tubes capable of attaining 2000 x *g*
- Rocking platform or rotator for decalcification at 37°C
- 0.5 M EDTA, pH 7.5
- Sterile, deionized water
- Optional: Saturated solution of ammonium oxalate, pH 3.0 to monitor the decalcification process

## 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).
- Vortexing should be performed by pulse-vortexing for 5–10 s.

## 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 37°C for use in step 3b or, with ≤100 mg bone, preheat to 56°C for use in step 7.

## Procedure

1. **Completely remove bone marrow and soft tissues using razor blades and/or sandpaper.**
2. **Crush the bone into small fragments. Grind to a fine powder using a metal blender half-filled with liquid nitrogen.**

   Alternatively, grind the bone to a fine powder using the TissueLyser and the Grinding Jar Set, S. Steel. See optimized protocol DY18, available at www.qiagen.com , for purification of total DNA from animal bones or teeth using the TissueLyser and the DNeasy Blood & Tissue Kit.
3. **If using ≤100 mg bone, follow step 3a; for >100 mg, follow step 3b.**

   The decalcification procedure in steps 3b to 6 is generally not necessary with small bone samples (≤100 mg) and may not be necessary for some larger bone samples.

**3a. Place up to 100 mg of the powdered bone into a 1.5 ml microcentrifuge tube. Proceed immediately with step 7.**

**3b. Transfer 100 mg – 5 g of the powdered bone into a sterile 50 ml polypropylene tube, and add 40 ml of 0.5 M EDTA, pH 7.5, to decalcify the sample. Agitate the tube on a rotator or rocking platform at 37°C for 24 h.**


4. **Centrifuge the sample at 2000 x *g* for 15 min. Discard the supernatant. Repeat the decalcification process several times.**

   **Note:** Generally, decalcification takes 3–5 days. The decalcification process can be monitored by adding a saturated solution of ammonium oxalate, pH 3.0, to the decanted supernatant. If the solution remains clear, the decalcification process can be stopped.

   Heat the rotator or rocking platform to 56°C after decalcification if it will be used for the incubation in step 7.

5. **Wash the pellet with 40 ml of sterile deionized water to remove ions that have accumulated during decalcification. Centrifuge the sample for 15 min at 2000 x *g* and discard the supernatant. Repeat this washing procedure 3 times.**
6. **Place up to 50 mg of the pellet into a 1.5 ml or 2 ml microcentrifuge tube.**
7. **Add 360 µl Buffer ATL and 40 µl proteinase K. Mix by vortexing, and incubate at 56°C until the pellet is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a shaking water bath or on a rocking platform.**
8. **Vortex for 15 s. Add 400 µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 400 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.

9. **Pipet up to 650 µl of the mixture from step 8 (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 reuse the collection tube.***
10. **Repeat step 9 until all of the sample has been loaded.**
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 ≥6000 x *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 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).

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


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 or water 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*).

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.

## 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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