Quick-Start Protocol April 2016

DNeasy® Blood & Tissue Kit

The DNeasy Blood & Tissue Kit (cat. nos. 69504 and 69506) can be stored at room temperature (15–25°C) for up to 1 year if not otherwise stated on label.

Further information

- *DNeasy Blood & Tissue Handbook*: **www.qiagen.com/HB-2061**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Redissolve any precipitates in Buffer AL and Buffer ATL.
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates.
- Equilibrate frozen tissue or cell pellets to room temperature.
- Preheat an incubator to 56°C.
- Refer to the handbook for pretreatment of fixed tissue, insect, bacterial or other material.

1a. **Tissue**: Cut tissue (≤10 mg spleen or ≤25 mg other tissue) into small pieces, and place in a 1.5 ml microcentrifuge tube. For rodent tails, use 1 (rat) or 2 (mouse) 0.4–0.6 cm lengths of tail. Add 180 µl Buffer ATL. Add 20 µl proteinase K, mix by vortexing and incubate at 56°C until completely lysed. Vortex occasionally during incubation. Vortex 15 s directly before proceeding to step 2.

1b. **Nonnucleated blood**: Pipet 20 µl proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 50–100 µl anticoagulant-treated blood. Adjust volume to 220 µl with PBS. Proceed to step 2.

Enzymatic lysis buffer:
- 20 mM Tris·Cl, pH 8.0
- 2 mM sodium EDTA
- 1.2% Triton® X-100
- Immediately before use, add lysozyme to 20 mg/ml

1. Harvest cells (maximum 2 x 109 cells) in a microcentrifuge tube by centrifuging for 10 min at 5000 x g (7500 rpm). Discard supernatant.
2. Resuspend bacterial pellet in 180 µl enzymatic lysis buffer.
3. Incubate for at least 30 min at 37°C.
After incubation, heat the heating block or water bath to 56°C if it is to be used for the incubation in step 5.
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4. Add 25 µl Proteinase K and 200 µl Buffer AL (without ethanol). Mix by vortexing.
Note: Do not add Proteinase K directly to Buffer AL. Ensure that ethanol has not been added to Buffer AL (see "Buffer AL", page 19). Buffer AL can be purchased separately (see ordering information starting on page 59).
5. Incubate at 56°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. Add 200 µl 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.
7. Continue with step 4 of the protocol "Purification of Total DNA from Animal Tissues Spin- Column Protocol)", page 30. [AKA step 4 here too]

~~1a. **Cultured cells**: Centrifuge a maximum of 5 x 10^6 cells for 5 min at 300 x g (1490 rpm). Resuspend in 200 µl PBS. Add 20 µl proteinase K. Proceed to step 2.~~

~~2. Add 200 µl Buffer AL. Mix thoroughly by vortexing. Incubate blood samples at 56°C for 10 min.~~

~~3. Add 200 µl ethanol (96–100%). Mix thoroughly by vortexing.~~

4. Pipet the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at ≥6000 x *g* (8000 rpm) for 1 min. Discard the flow-through and collection tube.

5. Place the spin column in a new 2 ml collection tube. Add 500 µl Buffer AW1. Centrifuge for 1 min at ≥6000 x *g*. Discard the flow-through and collection tube.

CHECK: has ethanol been added to AW1 already?

6. Place the spin column in a new 2 ml collection tube, add 500 µl Buffer AW2 and centrifuge for 3 min at 20,000 x *g* (14,000 rpm). Discard the flow-through and collection tube.

CHECK: has ethanol been added to AW2 already?

Throw out flow-through and re-centrifuge another minute in the same tube, just to make sure it's super dry. Needs to be dry so no ethanol remains.

7. Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube.

8. Elute the DNA by adding 200 µl Buffer AE to the center of the spin column membrane. Incubate for 1 min at room temperature (15–25°C). Centrifuge for 1 min at ≥6000 x *g*.

9. **Optional**: Repeat step 8 for increased DNA yield.

Don't do more than 200uL at a time in 1.5 tube, since bottom of spin tube will touch.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.