

DNeasy Protocol for Rodent Tails

- Before using the DNeasy Tissue Kit for the first time, please read “Technical Information” on page 8.
- 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† 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.
- 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 at $\geq 6000 \times g$.
- Vortexing should be performed by pulse vortexing for 5–10 sec.
- 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 11).

1. Cut one (rat) or up to two (mouse) 0.4–0.6-cm lengths of tail into a 1.5-ml microcentrifuge 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.

2. Add 20 µl 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 column. If the lysate still appears very gelatinous after incubation and vortexing, refer to the “Troubleshooting Guide” on page 29 for recommendations.

* Contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents that contain bleach.

† Contains sodium azide. Take appropriate laboratory safety measures, and wear gloves when handling.

Optional: Add 4 μ l of RNase A (100 mg/ml) to the sample, mix by vortexing, and incubate for 2 min at room temperature.

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 sec. Add 400 μ l Buffer AL–ethanol mixture (see “Important notes before starting” above) 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 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 column sitting 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 column in a new 2-ml collection tube (provided), add 500 μ 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 column in a new 2-ml collection tube (provided), add 500 μ l of Buffer AW2, and centrifuge for 3 min at full speed to dry the DNeasy membrane. Discard flow-through and collection tube.**

It is important to dry the membrane of the DNeasy mini 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. Discard flow-through and collection tube.

Following the centrifugation step, remove the DNeasy mini column carefully so that the column does not contact 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 full speed.)

- 7. Place the DNeasy mini column in a clean 1.5-ml or 2-ml microcentrifuge tube (not provided) and pipet 200 μ 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 μ l (instead of 200 μ l) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield (see Figure 3 on page 12).

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 μ l should not be eluted into a 1.5-ml microcentrifuge tube because the DNeasy mini column will contact the eluate.