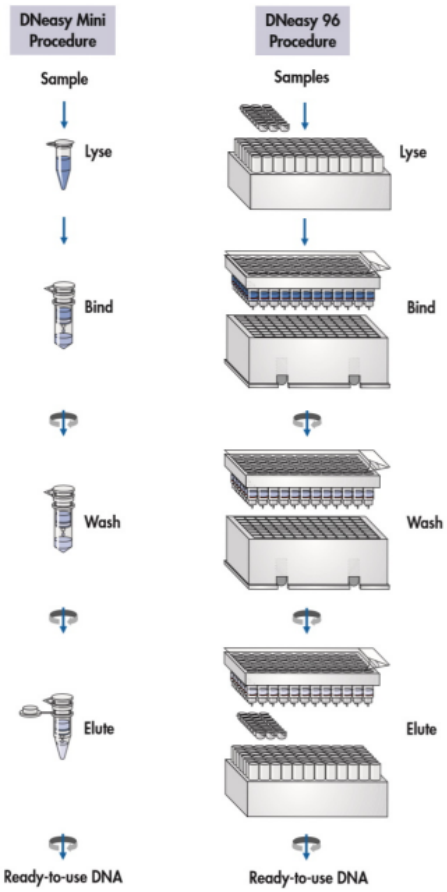
DNA Extraction Protocol

For DNA Extraction, we use Qiagen DNEasy Blood and Tissue Kits. You can view a walkthrough of the entire process here.

Kit Overview

DNeasy Blood & Tissue procedures are simple (see flowchart). Samples are first lysed using Proteinase K.* Buffering conditions are adjusted to provide optimal DNA-binding conditions and the lysate is loaded onto the DNeasy Mini spin column or the DNeasy 96 plate. During centrifugation, DNA is selectively bound to the DNeasy membrane as contaminants pass through. Remaining contaminants and enzyme inhibitors are removed in two efficient wash steps and DNA is then eluted in water or buffer, ready for use. DNeasy purified DNA has A260/A280 ratios of 1.7–1.9, and absorbance scans show a symmetric peak at 260 nm confirming high purity.



Kit Contents

DNeasy Blood & Tissue Kit Catalog no. Number of preps	(50) 69504 50	(250) 69506 250
DNeasy Mini Spin Columns (colorless) in 2 ml Collection Tubes	50	250
Collection Tubes (2 ml)	100	500
Buffer ATL	14 ml	50 ml
Buffer AL*	12 ml	2 x 33 ml
Buffer AW1 (concentrate)*†	19 ml	98 ml
Buffer AW2 (concentrate)†‡	13 ml	66 ml
Buffer AE	2 x 15 ml	2 x 60 ml
Proteinase K	1.25 ml	6 ml
Quick-Start Protocol	1	1

* Contains a chaotropic salt. Not compatible with disinfecting agents containing bleach. See page 7 for safety information.

† Buffer AW1 and Buffer AW2 are supplied as concentrates. Add ethanol (96–100%) according to the bottle label before use to obtain a working solution.

‡ Contains sodium azide as a preservative.

Other equipment needed

- Pipettes and pipette tips
- Vortexer
- Ethanol (96–100%)*
- Optional: RNase A (100 mg/ml; cat. no. 19101)
- 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 *** We use the benchtop incubator/oven
- Lab coat & safety gloves

Sample collection and Storage

Best results are obtained with fresh material or material that has been immediately frozen and stored at –90°C to –15°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor-quality starting material will also lead to reduced length and yield of purified DNA. After Proteinase K digestion, tissue samples can also be stored in Buffer ATL for up to 6 months at ambient temperature without any reduction in DNA quality. For certain bacterial cultures that accumulate large amounts of metabolites and/or form very dense cell walls, it is preferable to harvest cells in the early log phase of growth. Fresh or frozen cell pellets can be used in the procedure.

Notes before starting

- Perform all centrifuge steps at room temperature
- Redissolve any precipitates in Buffer AL and Buffer ATL
- Add ethanol (96–100%) to Buffers AW1 and A12 according to the bottle label before use to obtain a working solution.
    - Note: Buffer AW1 and Buffer AW2 are stable for at least 1 year after the addition of ethanol when stored closed at room temperature (15–25°C).
- Before starting, let all tissue samples come up to room temperature
- Preheat an incubator to 56C

Step 1: Tissue

- Cut tissue (<10 mg spleen, <25 mg other tissue) into small pieces (about the size of a pen mark)
    - Note: A 2 mm cube (approximately this size:) of most animal tissues weighs approximately 10–15 mg.
- Place into a 1.5 microcentrifuge tube
- Add 180 ul Buffer ATL
- Add 20 ul proteinase K. Mix by vortexing.
- Incubate at 56C until lysed
- Vortex for 15 seconds.

Step 2

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

Step 3

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

Step 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.

Step 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.

Step 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.

Step 7

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

Step 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.

Step 9 (Optional)

- Repeat step 8 for increased DNA yield.