 Protocol: Pretreatment for Gram-Positive Bacteria

This protocol is designed for purification of total DNA from Gram-positive bacteria, such as *Corynebacterium* spp. and *B. subtilis*. The protocol describes the preliminary harvesting of bacteria and incubation with lysozyme to lyse their cell walls before DNA purification.

**Important points before starting**

- See “Quantification of starting material”, page 17, for details of how to collect and store samples, and how to determine the number of cells in a bacterial culture.

- Ensure that ethanol has not been added to Buffer AL (see “Buffer AL”, page 18). Buffer AL can be purchased separately (see page 56 for ordering information).

- This pretreatment protocol has not been thoroughly tested and optimized for high-throughput DNA purification using the DNeasy 96 Blood & Tissue Kit. As a general guideline, we recommend to decrease the amount of starting material when using this protocol with the DNeasy 96 Blood & Tissue Kit.

**Things to do before starting**

- Prepare enzymatic lysis buffer as described in “Equipment and Reagents to Be Supplied by User”, page 14.

- Preheat a heating block or water bath to 37°C for use in step 3.

**Procedure**

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.

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 18). Buffer AL can be purchased separately (see page 56 for ordering information).

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.**
A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the DNeasy procedure. Some tissue types (e.g., spleen, lung) may form a gelatinous lysate after addition of Buffer AL and ethanol. In this case, vigorously shaking or vortexing the preparation is recommended.

4. Pipet the mixture from step 3 (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 collection tube.*

5. 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.*

6. 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).

7. 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 ≥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 Figure 2, page 21).

8. Recommended: For maximum DNA yield, repeat elution once as described in step 7.

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 7 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 page 8 for safety information.
For blood and cultured cells
- PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl)

For pretreatment of paraffin-embedded tissue (page 41)
- Xylene

For pretreatment of formalin-fixed tissue (page 43)
- PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl)

For pretreatment of gram-positive bacteria (page 45)
- 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