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DNeasy® Blood & Tissue Handbook

DNeasy Blood & Tissue Kit
DNeasy 96 Blood & Tissue Kit
For purification of total DNA from
animal blood
animal tissue
rodent tails
ear punches
cultured cells
fixed tissue
bacteria
insects

Sample to Insight

QIAGEN

Protocol: Purification of Total DNA from Animal Blood or Cells (Spin-Column Protocol)

This protocol is designed for purification of total DNA from animal blood (with nucleated or non-nucleated erythrocytes) or from cultured animal or human cells.

Important points before starting

- If using the DNeasy Blood & Tissue Kit for the first time, read "Important Notes" (page 15).
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.
- PBS is required for use in step 1 (see page 14 for composition). Buffer ATL is not required in this protocol.
- **Optional**: RNase A may be used to digest RNA during the procedure. RNase A is not provided in the DNeasy Blood & Tissue Kit (see "Copurification of RNA", page 20).

Things to do before starting

- Buffer AL may form a precipitate upon storage. If necessary, warm to 56°C until the precipitate has 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 56°C for use in step 2.

Procedure

1. For blood with non-nucleated erythrocytes, follow step 1a; for blood with nucleated erythrocytes, follow step 1b; for cultured cells, follow step 1c. Blood from mammals contains non-nucleated erythrocytes. Blood from animals, such as birds, fish or frogs, contains nucleated erythrocytes.

   1a. **Non-nucleated**: Pipet 20 µl Proteinase K into a 1.5 ml or 2 ml microcentrifuge tube (not provided). Add 50–100 µl anticoagulated blood. Adjust the volume to 220 µl with PBS. Continue with step 2.
   Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature (15–25°C) before continuing with step 2.

   1b. **Nucleated**: Pipet 20 µl Proteinase K into a 1.5 ml or 2 ml microcentrifuge tube (not provided). Add 5–10 µl anticoagulated blood. Adjust the volume to 220 µl with PBS. Continue with step 2.
   Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature before continuing with step 2.

   1c. **Cultured cells**: Centrifuge the appropriate number of cells (maximum 5 x 10^6) for 5 min at 300 x *g*. Resuspend the pellet in 200 µl PBS. Add 20 µl Proteinase K. Continue with step 2.
   When using a frozen cell pellet, allow cells to thaw before adding PBS until the pellet can be dislodged by gently flicking the tube.
   Ensure that an appropriate number of cells is used in the procedure. For cell lines with a high degree of ploidy (e.g., HeLa cells), it is recommended to use less than the maximum number of cells listed in Table 1 (page 16).
   Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing with step 2.

2. Add 200 µl Buffer AL (without added ethanol). Mix thoroughly by vortexing, and incubate at 56°C for 10 min.

   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).

   It is essential that the sample and Buffer AL are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.

3. 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.

4. Pipet the mixture from step 3 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* (14,000rpm).

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 6 for safety information

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 1min, 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 23).

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.