QIAGEN Supplementary Protocol:

Purification of DNA from cultured animal cells using the DNeasy® 96 Tissue Kit

This protocol is designed for the purification of DNA from cultured animal cells using the DNeasy 96 Tissue Kit.

IMPORTANT: Please consult the "Safety Information" and "Important Notes" sections in the *DNeasy 96 Tissue Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Equipment and reagents to be supplied by user

- Centrifuge 4-15C (cat. no. 81020) with Plate Rotor 2 x 96 (cat. no. 81031)
- Multichannel pipet with tips (see "Multichannel pipet and tips" in the *DNeasy 96 Tissue Handbook*)
- Reagent reservoirs for multichannel pipet
- Ethanol (96–100%)
- Incubator
- Phosphate-buffered saline (PBS)
- Optional: Trypsin solution (0.10–0.25% trypsin in PBS), see protocol step 1b
- Optional: Cell scraper, see protocol step 1b

Important points before starting

- This protocol requires undiluted Buffer AL. If the Buffer AL provided in the DNeasy 96 Tissue Kit has already been diluted with ethanol, more Buffer AL can be ordered from QIAGEN (cat. no. 19075).
- Ensure that ethanol has been added to Buffer AW1 and Buffer AW2 (see *DNeasy 96 Tissue Handbook*).
- Preheat an incubator or oven to 70°C.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 70°C for 5 minutes.
- Equilibrate samples to room temperature.
- All centrifugation steps are performed at room temperature.
- Avoid repeated thawing and freezing of stored samples since this will lead to reduced DNA size.

Procedure

1. Harvest cells according to steps 1a and 1b.

1a. Cells grown in suspension

Centrifuge the appropriate number of cells (max. 5 x 10^6 diploid cells) for 5 min at 300 x *g* in collection microtubes (provided). Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 2.

1b. Cells grown in monolayer

Cells grown in monolayer can be detached from the culture flask by either trypsinization or using a cell scraper (e.g., a rubber policeman).

Trypsinization

To trypsinize cells: Aspirate the medium and wash cells with PBS. Aspirate the PBS and add trypsin solution (0.10–0.25% trypsin in PBS). After cells have become detached from the dish or flask, collect the cells in medium, and transfer the appropriate number of cells (max. 5 x 10^6 diploid cells) to collection microtubes (provided). Centrifuge for 5 min at 300 x *g*. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 2.

Cell scraper

Using a cell scraper, detach cells from the dish or flask. Transfer the appropriate number of cells (max. 5 x 10^6 diploid cells) to collection microtubes (provided) and centrifuge for 5 min at 300 x *g*. Remove the supernatant completely and discard without disturbing the cell pellet. Continue with step 2.

2. Use the 96-Well-Plate Register provided to record the locations of the samples in the microtubes.

Mark the collection microtubes so that samples can be easily identified.

3. Resuspend cell pellet in PBS to a final volume of 200 µl.

Note: To avoid cross-contamination when sealing the microtubes with caps, do not touch the microtube rim with the pipet tip.

4. Add 20 µl Proteinase K solution to the collection microtubes, taking care not to wet the rims of the collection microtubes.

Note: RNA and DNA will be purified in parallel. If RNA-free genomic DNA is required, 20 µl of RNase A (20 mg/ml) should be added to the sample prior to the addition of Buffer AL in step 5.

5. Add 200 µl Buffer AL to each sample, taking care not to wet the rims of the microtubes. Seal the tubes using the collection microtube caps provided.

6. **Mix thoroughly by shaking the racked microtubes vigorously for 15 s.**

   For efficient lysis, it is essential that the samples and Buffer AL are mixed immediately and thoroughly to yield a homogeneous solution. Hold the collection microtube block with both hands and shake up and down vigorously.

   Note: Simply inverting the block several times is not sufficient to initiate efficient lysis. Similarly, vortexing or placing the plate on a shaker is not adequate.

7. **Centrifuge the samples briefly at 3000 rpm (~1450 x *g*) to collect any solution from the caps.**

   Allow the centrifuge to reach 3000 rpm (~1450 x *g*), and then stop the centrifuge.

   Note: When processing 96 samples using a single collection microtube rack, the centrifuge must be accurately balanced.

8. **Incubate the samples at 70°C for at least 10 min in an incubator or oven.**

   Longer incubation at 70°C has no effect on the quality of the purified DNA.

   Note: Place a weight on top of the caps during the incubation.

9. **Centrifuge briefly at 3000 rpm (~1450 x *g*) to collect any lysate from the caps.**

   Allow the centrifuge to reach 3000 rpm (~1450 x *g*), and then stop the centrifuge.

10. **Remove the caps and add 200 µl ethanol (96–100%) to each collection microtube.**
11. **Seal the collection microtubes using new caps (provided). Shake the collection microtubes vigorously for 15 s.**
12. **Centrifuge briefly at 3000 rpm (~1450 x *g*) to collect any solution from the caps.**

    Allow the centrifuge speed to reach 3000 rpm (~1450 x *g*), and then stop the centrifuge.

13. **Place a DNeasy 96 plate on top of an S-block. Mark the plate for later identification.**
14. **Carefully apply the mixture from step 12 (620 µl per well) to the DNeasy 96 plate.**

    Take care not to wet the rims of the wells to avoid aerosol formation during centrifugation.

    Note: Lowering pipet tips to the bottoms of the wells of the collection microtubes may cause sample overflow and cross-contamination. Therefore, remove one set of caps at a time, and begin drawing up the samples as soon as the pipet tips contact the liquid. Repeat until all the samples have been transferred to the DNeasy 96 Plate wells.

15. **Seal the DNeasy 96 plate with an AirPore tape sheet. Centrifuge at 6000 rpm (~5790 x *g*) for 4 min.**

    If lysate remains in any of the wells, centrifuge for a further 10 min.

16. **Remove the AirPore tape sheet. Carefully add 500 µl Buffer AW1 to each well.**
17. **Seal the DNeasy 96 plate with a new AirPore tape sheet. Centrifuge at 6000 rpm (~5790 x *g*) for 2 min.**
18. **Remove the AirPore tape sheet. Carefully add 500 µl Buffer AW2 to each well.**

19. **Centrifuge at 6000 rpm (~5790 x *g*) for 15 min.**

    The heat generated during centrifugation ensures evaporation of residual ethanol in the sample (from Buffer AW2) that might otherwise inhibit downstream reactions.

20. **Place the DNeasy 96 plate on top of a rack of elution microtubes (provided).**

21. **To elute DNA, add 200 µl Buffer AE or distilled water, equilibrated to room temperature, to each well using a multichannel pipet. Seal the DNeasy 96 plate with a new AirPore tape sheet and incubate for 1 min at room temperature. Centrifuge at 6000 rpm (~5790 x *g*) for 4 min. Seal the wells of the microtubes for storage using the caps for elution microtubes provided.**

    DNA yields can be increased by incubating the DNeasy 96 plate loaded with Buffer AE for 5 min at room temperature before centrifugation.

    A second elution step with a further 200 µl Buffer AE increases yields by up to 20%. Elution with volumes of less than 200 µl significantly increases the final DNA concentration, but slightly reduces the overall yield. For samples containing less than 1 µg DNA, elution in 50 µl Buffer AE or water is recommended. Eluting with 2 x 100 µl instead of 1 x 200 µl does not increase elution efficiency. For long-term storage of DNA, eluting in Buffer AE and storing at –20°C is recommended, since DNA stored in water is subject to acid hydrolysis.