

**User-developed
protocol**

User-Developed Protocol:

Isolation of genomic DNA from saliva using the DNeasy[®] Tissue Kit; spin procedure

This procedure has been adapted by customers from the DNeasy[®] Tissue Protocols, and is for use with the DNeasy Tissue Kit. **It has not been thoroughly tested and optimized by QIAGEN.**

Please be sure to read the QIAGEN[®] *DNeasy Tissue Kit Handbook* and the detailed Animal Tissues Protocol carefully before beginning this procedure.

Important notes before starting

- Ensure that Buffer AL, Buffer AW1, and Buffer AW2 have been prepared according to the *DNeasy Tissue Kit Handbook*.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 70°C.
- All centrifugations are carried out at room temperature.

Procedure

1. Collect 1 ml saliva.

Note: Ensure that the animal from which the sample was taken has not eaten any food in the 30 min prior to sample collection.

2. Add 4 ml PBS (not provided) to the sample and centrifuge at 1800 x g for 5 min.

3. Carefully decant the supernatant. Resuspend the pellet in 180 µl PBS.

DNeasy Spin Columns copurify RNA and DNA in parallel when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not the PCR itself. If RNA-free genomic DNA is required, 20 µl of an RNase A stock solution (20 mg/ml) should be added to the sample prior to the addition of Proteinase K.

4. Add 25 µl Proteinase K solution and 200 µl Buffer AL to the sample, mix thoroughly by vortexing, and incubate at 70°C for 10 min. Continue with step 3 of the Cultured Animal Cells Protocol in the *DNeasy Tissue Kit Handbook*.

In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed immediately and thoroughly.

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