Extraction method A (FMS and CR)

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Abstract

This protocol allows for adequate DNA extraction from fresh tissue samples.

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Before start

Separate PCR-free facility

Materials

- Buffer AW1 19081 by Qiagen
- Buffer AW2 19072 by Qiagen
- Buffer AE 19077 by Qiagen
- Buffer ATL 19076 by Qiagen
- ✓ Ethanol by Contributed by users

Protocol

Extraction

Step 1.

Cut tissue into small pieces.

Extraction

Step 2.

Digestion of ca. 25 mg sample with Buffer ATL (Qiagen, Hilden, DE) and Proteinase K overnight at 56 °C.

AMOUNT

25 mg Additional info: Sample

NOTES

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Incubation was performed on a thermomixer with 800 rpm

Extraction

Step 3.

After pelleting remaining material, remove supernatant and added to Buffer AL and ethanol.

Extraction

Step 4.

Transfer the solution to DNeasy Mini spin column (Qiagen, Hilden, DE) and centrifuge at 8000 rpm.

Extraction

Step 5.

DNA purification following manufacturer's protocol with Buffer AW1 and Buffer AW2 (Qiagen, Hilden, DE).

Extraction

Step 6.

Prior to elution, incubate DNA in the membrane with elution Buffer AE for 5 min at room temperature.

Extraction

Step 7.

Elution proceeds by centrifugation at 8000 rpm for 1 min.

Extraction

Step 8.

Measure DNA concentration on Nanodrop (Thermo Fischer Scientific, Darmstadt, DE).