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Extraction Method D (PRP and RM)

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Keywords: adequate dna extraction from archival sample, dna extraction, adequate dna extraction, dna, archival sample, prp, rm

Abstract

This protocol allows for adequate DNA extraction from archival samples.

Materials

MATERIALS

⊗ Buffer AL **Catalog #19075**

⊗ Buffer AW1 **Qiagen Catalog #19081**

⊗ Buffer AW2 **Qiagen Catalog #19072**

⊗ Buffer AE **Qiagen Catalog #19077**

⊗ Buffer ATL **Qiagen Catalog #19076**

⊗ Ethanol

Before start

Separate PCR-free facility

Extraction

- 1 Digestion of ca. 50 mg sample with Buffer ATL (Qiagen, Hilden, DE) and Proteinase K overnight at 56 °C.

 50 µL Buffer ATL

Note

Incubation was performed with rotation of the samples in an oven.

- 2 After pelleting remaining material, remove the supernatant and add to Buffer AL and ethanol.
- 3 Transfer the solution to DNeasy Mini spin column (Qiagen, Hilden, DE)
- 4 Centrifuge the solution at 8000 rpm.
- 5 DNA purification following manufacturer's protocol with Buffer AW1 and Buffer AW2 (Qiagen, Hilden, DE).
- 6 Prior to elution, incubate DNA in the membrane with elution Buffer AE for 20 min at 37 °C.

 00:20:00 Incubation at 37 °C
- 7 Proceed elution by centrifugation at 8000 rpm for 1 min.

 00:01:00 Centrifugation
- 8 Prior to library construction, analyze small aliquots of each extract on Nanodrop (Thermo Fischer Scientific, Darmstadt, DE) for estimation of DNA concentration.