Extraction method B (FMS and CR)

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Abstract

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

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Before start

Separate PCR-free facility

Materials

- Buffer AL 19075 by Contributed by users
  Buffer AW1 19081 by Qiagen
  Buffer AW2 19072 by Qiagen
  Buffer AE 19077 by Qiagen
- Proteinase K by Contributed by users
- Ethanol by Contributed by users
- PBS by Contributed by users

Protocol

Extraction

Step 1.

Digestion of ca. 100 µl sample with Buffer ATL (Qiagen, Hilden, DE), Proteinase K and PBS for 10 min at 56 °C.

AMOUNT
100 µl Additional info: Sample

NOTES

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Incubation was performed on a thermomixer with 800 rpm

Extraction

Step 2.

Add ethanol.

Extraction

Step 3.

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

Extraction

Step 4.

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

Extraction

Step 5.

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

Extraction

Step 6.

Elution proceeds by centrifugation at 8000 rpm for 1 min.

Extraction

Step 7.

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