DNA Extraction of Placenta Tissue

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WORKS FOR ME 1

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ABSTRACT

This protocol describes extracting DNA from placenta tissue, starting with dissociation.

PROTOCOL CITATION

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MATERIALS TEXT

1.0 mm diameter zirconia/silica beads, BioSpec Products, catalog 11079110z
DNeasy Blood and Tissue Kit, Qiagen, catalog 69504
100% ethanol
Nuclease-free water
2 mL screw-cap tubes with O-ring gaskets
Nuclease-free low-retention 1.5 mL microcentrifuge tubes

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2h

Tissue Dissociation and Lysis

1 Place placenta tissue samples in screw cap tubes with O-ring gaskets on ice to thaw.

2 Label a 1.5 mL microcentrifuge tube for each placenta sample. Aliquot 1 mL silica beads per tube.

3 Add 540 uL Buffer ATL and 20 uL Proteinase K to each tissue. Pour in prepared beads.

4 Tightly close tubes. Place tubes in Bead Beater, and tightly close screw-on parts. Homogenize for 2 minutes.

5 Incubate lysates with beads in 56C water bath or heat block for 2 minutes.

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6 Transfer lysate to pre-labeled microcentrifuge tubes. Do not discard beads.

7 Add 600 uL Buffer AL to screw-cap tubes with beads. Vortex and quick spin. Transfer supernatant to microcentrifuge tube with lysate. Discard screw-cap tube with beads.

8 Vortex lysate until homogenized.

9 Incubate lysates 56C for 10 minutes. Quick spin.

10 Freeze at -80C or proceed with DNA extraction.

DNA Extraction

1h 30m

11 Thaw lysates at room temperature.

12 Prepare columns and buffers. Add 25 mL 100% ethanol to Buffer AW1 and 30 mL 100% ethanol to Buffer AW2.

13 Add one-half volume (approximately 500 uL) 100% ethanol to lysate. Mix thoroughly by vortexing.

14 Pipet maximum 700 uL of the mixture to column. Centrifuge at >=6000g for 1 minute. Discard flow through. Repeat this step until all lysate has passed through column.

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15 Add 500 uL Buffer AW1. Centrifuge for 1 minute at >=6000g. Discard flow-through and collection tube.

16 Place the spin column in a new 2 mL collection tube. Add 500 uL Buffer AW2. Centrifuge for 3 minutes at 20000g. Discard flow-through and collection tube.

16.1 While spinning, label 1.5 mL microcentrifuge tubes for elution.

17 Transfer column to pre-labeled microcentrifuge tubes.

18 Elute DNA by adding 100 uL Buffer AE or nuclease-free water to the center of the membrane. Incubate for 1 minute at room temperature. Centrifuge for 1 minute at >=6000g.

19 Repeat step 18.

20 Store eluted DNA at -20C.

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