

COMMENTS 0



ONA Extraction of Placenta Tissue

This protocol is published without a DOI.

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WORKS FOR ME

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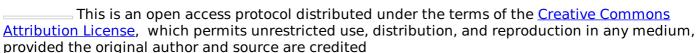
ABSTRACT

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

PROTOCOL CITATION

Cayla Mason 2022. DNA Extraction of Placenta Tissue . protocols.io https://protocols.io/view/dna-extraction-of-placenta-tissue-cjejujcn

LICENSE



CREATED

Nov 16, 2022

LAST MODIFIED

Nov 16, 2022

PROTOCOL INTEGER ID

72875



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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Tissue Dissociation and Lysis

2h

- 1 Place placenta tissue samples in screw cap tubes with 0-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.
- Add 540 uL Buffer ATL and 20 uL Proteinase K to each tissue. Pour in prepared beads.
- 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.

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

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.

Vortex lysate until homogenized.

Incubate lysates 56C for 10 minutes. Quick spin.

Freeze at -80C or proceed with DNA extraction.

DNA Extraction

- 11 Thaw lysates at room temperature.
- Prepare columns and buffers. Add 25 mL 100% ethanol to Buffer AW1 and 30 mL 100% ethanol to Buffer AW2.

1h 30m

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