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We use this protocol and it's working

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High Molecular Weight DNA Extraction Protocol [Tissue Sample]

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ABSTRACT

This is an organic extraction protocol used for high molecular DNA extraction for tissue samples.

High Molecular Weight DNA Extraction Protocol

6h 40m 10s

1 **Lysis Tissue Sample**

Add 500 µL of lysis buffer to 50 - 150 mg of Sample of sample. Make sure the tissue sample has been finely cut.

2 **Denatures and digest proteins that are subsequently hydrolyzed with Proteinase K** 10s

Add 15 µL of Proteinase K and vortex for 00:00:10 .

3 **Incubation Step** 2h 30m

Incubate on a shaking incubator at 55 °C (02:30:00 or till dissolved).

4 **Remove RNA with RNAse**

Add 10 µL of RNAse and vortex briefly.

5 **Incubation Step** 10m

Incubate in a shaking incubator for 00:10:00 at 37 °C .

6 **Partitioning of lipids and debris into an organic phase using P:C:IA**

Add 525 µL of P:C:IA (25:24:1). Vortex until emulsion is formed.

7 **Centrifugation Step**

Centrifuge at 10000 x g, Room temperature , 00:10:00

8 **Acquiring DNA in the Aqueous Phase**

Pipette 400 µL of the aqueous layer into a new tube.

9 **Neutralize the charges on the sugar-phosphate backbone of the DNA with Sodium Acetate**

Add 1/10th (20 µL) 3M Sodium Acetate pH 5.2 . Vortex gently (avoid creating bubbles).

10 **Precipitation Step**

Add 2 vol (440 µL) of ice-cold 100% Ethanol. Mix gently and slowly by inverting the tube.

11 **Incubation Step** 2h

Incubate at -20 °C for 02:00:00 .

12 **Centrifugation Step**

Centrifuge at 10000 x g, Room temperature , 00:20:00 . Discard supernatant carefully.

13 **Wash Step**

Wash with 500 µL of ice cold 70% ethanol.

14 **Centrifugation Step**

Centrifuge 10000 x g, 00:05:00

15 Repeat Wash Step (Step 17 and 18) 2 more times.

16 Quick Spin the tube one more time and pipette any remaining ethanol out of the tube.

17 **Dry** 2h

Air dry for at least 02:00:00

18 **Final Elution**

Add 35 µL of elution buffer (EB) and gently mix by tapping.

19 **Storage**

Store at 4 °C .