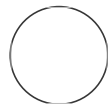




JAN 03, 2024

## High Molecular Weight DNA Extraction Protocol [Tissue Sample]

Aswini Leela<sup>1</sup><sup>1</sup>Monash University Malaysia Genomics Platform

Aswini Leela

### ABSTRACT

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

OPEN  ACCESS

**Protocol Citation:** Aswini Leela 2024. High Molecular Weight DNA Extraction Protocol [Tissue Sample]. **protocols.io**  
<https://protocols.io/view/high-molecular-weight-dna-extraction-protocol-tiss-c6xtzfnn>

**License:** This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working  
We use this protocol and it's working

**Created:** Jan 03, 2024


**Last Modified:** Jan 03, 2024

**PROTOCOL integer ID:**  
92883

# High Molecular Weight DNA Extraction Protocol



6h 40m 10s

## 1 Lysis Tissue Sample

Add  500  $\mu\text{L}$  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  $\mu\text{L}$  of Proteinase K and vortex for  00:00:10 .

## 3 Incubation Step

2h 30m



Incubate on a shaking incubator at  55  $^{\circ}\text{C}$  (  02:30:00 or till dissolved).

## 4 Remove RNA with RNase


Add  10  $\mu\text{L}$  of RNase and vortex briefly.

## 5 Incubation Step


10m

Incubate in a shaking incubator for  00:10:00 at  37  $^{\circ}\text{C}$  .


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

Add  525  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  ) 3M Sodium Acetate  5.2 . Vortex gently (avoid creating bubbles).

## 10 Precipitation Step

Add 2 vol (  440  $\mu\text{L}$  ) of ice-cold 100% Ethanol. Mix gently and slowly by inverting the tube.

## 11 Incubation Step

Incubate at  -20 °C for  02:00:00 .

2h

## 12 Centrifugation Step

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

## 13 Wash Step

Wash with  500  $\mu\text{L}$  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

Air dry for at least  02:00:00

2h

## 18 Final Elution

Add  35  $\mu\text{L}$  of elution buffer (EB) and gently mix by tapping.

## 19 Storage

Store at  4  $^{\circ}\text{C}$  .