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# CTAB DNA Extraction Protocol for Mollusks

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**ABSTRACT** 

This is a comprehensive CTAB extraction protocol intended for DNA extraction of mollusks. It includes detailed methodology and several reagent preparation.

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Method

1

Slice tissue sample using a clean, sterile scalpel. Put sample into a 1.5 ml tube with

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```
⊠CTAB extraction
    ■400 µL solution Teknova Catalog #C2190
2
    Add ■10 µL Seproteinase K Contributed by users M20 mg/mL and invert tube to
    mix.
3
    Incubate at § 50 °C for 60 minutes until lysed (or longer depending on tissue thickness).
    Invert tube occasionally to mix sample.
4 Add ⊒400 µL
    Organics Catalog #327155000
                                                                          . Gently
    shake tube to emulsify sample.
5
    Leave at room temperature for 2 minutes and shake tube to mix.
6
    Spin tube at 13 000 rpm for 2 minutes and transfer upper layer to a 1.5 ml tube.
7
   Add $\bigsigma 5 \mu L \text{ of } \infty RNase A Contributed by users \text{[M]10 mg/mL} \text{. Leave for 30 minutes at }
     8 37 °C .
8
    Add 1 equal volume of
    Organics Catalog #327155000
```

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2

Spin tube at 13 000 rpm for 2 minutes and transfer upper layer to a new 1.5 ml tube containing

■900 µL CTAB dilution solution. Gently invert tube to mix. Leave in fridge overnight (optional). 10 Spin tube at 13 000 rpm for 10 minutes. 11 Discard supernatant with a micropipette and add 1000 µL 0.4 M NaCl in TE. Invert tube to wash. 12 Spin tube at 13 000 rpm for 5 minutes and remove supernatant. 13 Add 300 µL 1.42 M NaCl in TE to dissociate the CTAB from the DNA. Invert tube until pellet becomes transparent. May require gentle shaking at § 37 °C 14 Add □600 µL ⊠ Ethanol Contributed by users (previously left at § -20 °C). Invert manually until precipitation appears. Leave precipitating overnight for a higher DNA concentration 15 Centrifuge at full speed for 8 minutes, then remove supernatant. Dry at room temperature or in heat block at § 37 °C

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Resuspend in  $\blacksquare 50~\mu L$  EB buffer. Use lesser volume of EB buffer if a higher concentration is required.

#### Notes

17

All centrifugations were performed using a benchtop centrifuge (Eppendorf - 5415 D)

18

The Nucleic acid quality is measured using a Nanodrop, and the A260/A230 ratio of 1.8-2.0 indicates good DNA purity. Qubit can be used to determine nucleic acid concentration.

19

Store DNA sample at -20°C until further use.

### Reagent preparation

20

**CTAB Extraction Solution** 



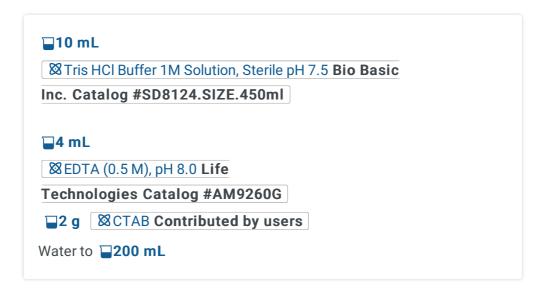
20.1

**CTAB Dilution Solution** 



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20.2

#### 0.4M Nacl in TE



20.3

## 1.42 M NaCl in TE

```
■24.8 mL 5M ⊠NaCl Contributed by users
■1 mL

⊠Tris HCl Buffer 1M Solution, Sterile pH 7.5 Bio Basic
Inc. Catalog #SD8124.SIZE.450ml
```

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