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RNA extraction protocol for the *Bungarus multicinctus* by using TRIZOL reagent (Invitrogen) V.1

In 1 collection

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ABSTRACT


RNA was extracted from the muscle of a *Bungarus multicinctus* using the TRIzol reagent (Invitrogen, USA) and RNA extraction kit (Thermo Scientific, USA) in this protocol.

RNA extraction

1h 5m 15s

- 1 The laboratory and test bench for RNA extraction were sterilized by alcohol and ultraviolet for 30 minutes.

30m



 00:30:00

All the equipment used in the experiment, such as mortar and pestle, were sterilized at high temperature one day before the experiment and dried in an oven.

Masks and gloves should be worn throughout the RNA extraction process. Avoid exposure of RNases to experimental samples and cause RNA degradation.


- 2 On the clean bench, take 50 mg of scented glands and put them into a pre-cooled mortar to ensure that the liquid nitrogen has never passed the sample, quickly grind the sample into powder, add 1 ml TRIZOL, mix the tissue powder with TRIZOL, and put it into In a centrifuge tube, stand at room temperature for 15 min.

15m


 Room temperature  00:15:00

- 3 Add 200 ml of chloroform, shake the centrifuge tube for 15 s to mix the chloroform and TRIZOL well.
Stand at room temperature for 10 min, put it in a refrigerated centrifuge and centrifuge at 4°C, 12000r/min, 15 min.

10m 15s


 00:00:15

 Room temperature  00:10:00

 12000 rpm, 4°C, 00:15:00

- 4 After centrifugation, the solution was separated into three phases. Take the upper aqueous phase into a new centrifuge tube, add three times the volume of ethanol to mix, stand at room temperature for 10 min.


10m

 Room temperature  00:10:00

- 5 Add the mixed solution to the purification column, centrifuge at 12000r/min at 4°C for 2 min, repeat three times to ensure that ethanol is removed.

 12000 rpm, 4°C, 00:02:00

- 6 RNA was purified with RNA extraction kit (Thermo Scientific, USA), and RNA was eluted with DEPC.

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- 7 Add RNase inhibitors to ensure that the RNA will not be degraded, and mark the outside of the centrifuge tube.

Check RNA purity and concentration, and confirm RNA integrity by electrophoresis.