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RNA Isolation for Tissue using TRIzol V.1

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Protocol status: In development
We are still developing and optimizing this protocol

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1 Add 1 mL TRIzol per 30 mg tissue and homogenize using handheld homogenizer.

2 Incubate at 25 °C for 00:05:00 to allow nucleoprotein complexes to dissociate.

3 Add 200 µL chloroform ([M] 20 % volume TRIzol) carefully and vortex to mix well.

4 Centrifuge at max speed for 00:15:00 at 4 °C .

5 Carefully remove the top aqueous phase and transfer to a new Eppendorf tube. The interphase and bottom organic phase can be saved for DNA and protein respectively.

6 Add 500 µL 100% isopropanol to the aqueous phase, mix by inversion and incubate at -20 °C for a minimum of 02:00:00 .

7 Spin down at maximum speed for 30 mins to precipitate RNA.

8 Remove supernatant, and add 1 mL of [M] 75 % volume ethanol to wash pellet.

9 Spin down at max speed for 15 minutes and remove supernatant.

10 Resuspend pellet in appropriate volume of nuclease free water.