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Sigma GeneElute total RNA extraction from fungal tissue

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We use this protocol and it's working

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Abstract

RNA extraction from fungal tissue using the Sigma GeneElute total RNA kit.

Troubleshooting

Tissue and workspace preparation

15m

1 ALL WORK MUST BE DONE IN THE STERILE HOOD THAT HAS BEEN CLEANED FOR RNA WORK

REGULARLY CHNAGE GLOVES AND WASH HANDS WITH RNASE TO AVOID DEGREDATION

MAKE SURE ALL PIPETORS, TIPS, AND ANY OTHER EQUIPMENT ARE IN THE HOOD AND CLEANED WITH RNASE

DO NOT MOVE ANYTHING IN OR OUT WITHOUT WASHING WITH RNASE

Reagents that need to be made:
Fresh, day of 70% etoh

2 Flash Freeze tissue in Liquid Nitrogen (LN2).

3 Grind Fungal Tissue to a fine powder with a mortar and pestle with LN2, be careful to not allow the tissue to thaw during this Process.

3.1 At this point frozen ground tissue can be stored at -80C for later extraction.

RNA extraction

15m

4 add 600ul of buffer RL to a 1.7ml eppendorf tube.

5 Place **no more than 50mg** of ground tissue into the sample tube with buffer RL.

6 Incubate for 15-30 minutes at room temperature Room temperature 15m

7 Centrifuge at maximum speed for 2 minutes

Equipment

Centrifuge	NAME
Benchtop Centrifuge	TYPE
Eppendorf	BRAND
5405000441	SKU
https://online-shop.eppendorf.us/US-en/Centrifugation-44533/Centrifuges-44534/Centrifuge-5425-PF-243560.html	LINK
Any benchtop centrifuge will suffice	SPECIFICATIONS

8 Transfer the lysate to a new RNAse free 1.7ml tube.

9 Add 1:1 volume (so 600ul) of **freshly** prepared 70% etoh

10 Vortex for 10 seconds, or until homogenous

11 Assemble a column with a waste tube from the GeneElute kit.

12 Add 600ul of lysate/etoh solution to the assembled column

13 Centrifuge at 6000G for 2 minuts

14 If lysate is not completely passes, spin at an additional 14,000G for 1 minute

15 Discard waste from waste tube, and repeat step 13 and 14 if more lysate/etoh exists.

16 Place column into a new waste collection tube

17 Add 400ul of wash solution A to column

18 Centrifuges at max speed for 1.5 minutes

19 discard waste from collection tube

20 Repeat steps 17-19 two more times (for a total of 3x wash steps)

21 Centrifuge Sample for a final 2 minute dry spin at max speed

22 Place column in a clean eppendorf tube provided in the GeneElute kit.

23 add 50ul of elution solution A

24 Centrifuge at 200G for 2 minutes

25 centrifuge at 14,000G for 1 minute

26 If the entire 50ul has not eluted, then spin for an additional minute at 14,000G

Quality control of RNA and storage

15m

27 Keep eluted samples on Ice, Prepare a bleach gel for RNA analysis

28 BLEACH GEL:

100ml TAE
400ul concentrated bleach
1g agarose

Make sure to add bleach **before** microwaving.

29 Load and run samples normally at 120V for ~20 minutes with a 100bp ladder.

30 immediately store samples at -80C after loading gel.