PicoPure RNA Isolation Kit

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2023-06-13

Note before starting:
- Pre-heat block to 42C
- Pre-make DNAse treatment master mix (1.1n)

Component	Volume	MM volume
DNAse I Stock	5 μL	
DNA Disgestion Buffer	35 μL	

Protocol

1. Extract cells with 100 μL of Extraction Buffer (XB)
   - Resuspend the cell pellet gently by pipetting
   - DO NOT VORTEX

2. Incubate at 42C for 30min

3. Centrifuge sample at 3000xg for two minutes

4. Collect supernatant
   - Can stop here and freeze RNA at 70C

5. Pre-condition the RNA Purification Column
   - Add 250 μL Conditioning Buffer onto the purification column filter membrane
   - Incubate RNA Purification Column with CB for 5min at RT
   - Centrifuge purification column at 16000xg for 1 min

6. Pipette 100 μL of 70% Ethanol (EtOH) into cell extract
   - Mix well by pipetting
   - DO NOT VORTEX

7. Add cell extract to column

8. Centrifuge column for 2 minutes at 100 x g and then 16000 x g for 30s

9. Pipette 100 μL Wash Buffer 1 (W1) into the purification column and centrifuge for 1min at 8000 x g

10. Add 40 μL of DNAse treatment master mix to purification tube

- Add directly onto membrane

11. Incubate at RT for 15 min

12. Pipette 40 μL Wash Buffer 1 (W1) onto purification column membrane and centrifuge at 8000 x g for 15 seconds

13. Pipette 100 μL Wash Buffer 1 (W2) into purification column membrane and centriguge for one minute at 8000 x g

14. Pipette 100 μL Wash Buffer 1 (W2) into purification column membrane and centriguge for two minutes at 16000 x g
    - Check if any wash buffer remains in column and centrifuge again at 16000 x g for one minute to clear all the liquid

15. Transfer purification column to new 0.5mL tube
    - Insert purification column/0.5mL assembly into 1.5mL tube for centrifugation

16. Pipette EB directly onto membrane of the purification column
    - Gently touch tip to surface of membrane to ensure maximum absorption of EB into membrane
    - Use 11 μL to 30 μL EB

17. Incubate purification column for one minute at room temperature

18. Centrifuge column for one minute at 1000 x g to distribute EB in column

19. Centrifuge column for one minute at 16000 x g to elute RNA

20. Start RT immediately or store at -80C until use