RNA Direct Lysis Method

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[1]In-house protocol

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1 Works for me This protocol is published without a DOI.

Eadewunm

ABSTRACT

RNA Direct Lysis method
(from Masse and Gottesman. 2003 Genes and Development. 17:2374-2383) (notes and italics are from E. Fozo by Selene Hess)

PROTOCOL CITATION

Elizabeth Fozo 2020. RNA Direct Lysis Method. **protocols.io** https://protocols.io/view/rna-direct-lysis-method-bpwrmpd6

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GUIDELINES

Direct Lysis solution:

- 320 mM NaAcetate
- 8% SDS
- 16 mM EDTA

N.B. Direct lysis solution will not stay in solution at RT. Prior to use, must get into solution by placing at 37 °C.

The recipe must be altered for use from minimal media.

ABSTRACT

RNA Direct Lysis method
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Steps

1 Grow cells to desired OD.

2 At desired OD, remove 750 ml of cells to a tube at 65°C containing 500 ml acid:phenol-chloroform with 100 ml of direct lysis solution (**SET-UP at least 25 minutes prior to harvesting to ensure the components are at 65°C**). Vortex to mix, and place the tube back at 65°C for 10-15 minutes.

Can store at -80 indefinitely. Once move on, can't stop till step 7, ethanol.

3 Spin 13000 r.p.m., **room temperature** for 5-10 minutes.

(15-20 minutes if from -80 storage)

4 Transfer supernatant to a new tube containing 500 ml of **65°C** acid phenol: chloroform. Spin as in step 3 *(RT)*.

5 Transfer supernatant and extract with 400 ml "regular" phenol: chloroform. *Gentle vortex*. Spin as in step 3 *(RT)*. May need to do additional extractions at this point.

6 Transfer supernatant to a new tube and extract with 400 ml chloroform. Spin as in step 3 (*RT*).

N.B. Liz and Aixia are the only ones who do this step, so it is clearly optional!
In interface is thick, white goo.

7 Transfer supernatant and add 700 ml 99% ethanol (*95% is fine*). Place in -80 C for 20 minutes (*or indefinitely*).

8 Spin 13000 r.p.m. **4°C**, 30 minutes. Wash with *400uL* 70% ethanol. Let pellet air dry.

9 Resuspend in RNase free water (*20-25uL*).

10 *Let sit for 10 minutes at RT and finish resuspending by pipetting up and down/flicking tube.*