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Phenol-chloroform extraction and ethanol precipitation of RNA

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1 Works for me dx.doi.org/10.17504/protocols.io.rzvd766

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ABSTRACT

Based on the protocol form NEB HiScribeTM T7 High Yield RNA Synthesis Kit

EXTERNAL LINK

https://www.neb.com/-/media/catalog/datacards-or-manuals/manuale2040.pdf

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

New England Biolabs HiScribeTM T7 High Yield RNA Synthesis Kit Instruction Manual

DOI

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PARENT PROTOCOLS

In steps of
hyRAD RNA probes preparation and capture
hyRAD RNA probes preparation and capture

MATERIALS

NAME	CATALOG #	VENDOR
Ethanol 100%		
Ethanol 70% [Note: freshly prepared]		
nuclease free water		
sodium acetate 3M solution		
EDTA 0.1 mM		

1 Adjust the reaction volume to 180 µl by adding 160 µl nuclease-free water. Add 20 µl of 3 M sodium acetate, pH 5.2 or 20 µl of 5 M ammonium acetate (1/10 of the total volume), mix thoroughly.

2 Extract with an equal volume (200 µl) of 1:1 phenol/chloroform mixture. Vortex and centrufugate at maximum speed for 5 min.

3 Collect the aqueous (upper) phase and transfer to a new tube.

4 Repeat steps 2 and 3.

5 Precipitate the RNA by adding 2 volumes of -20°C 100% ethanol. Incubate at –20°C or on dry ice for at least 30 minutes.

6 Collect the pellet by centrifugation at 4°C for 30 min.

7 Remove the supernatant and rinse the pellet with 500 µl of cold 70% ethanol.

8 Quick spin and collect the last drop of ethanol.

9 Dry the sample.

10 Resuspend the RNA in 50 µl of 0.1 mM EDTA. Store the RNA at –20°C or below.

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