

RNA clean-up by phenol:chloroform

Daniel Richter

Abstract

Phenol: chloroform extraction to clean up RNA (e.g. to remove DNase or DNase Inactivation Reagent.)

Based on phenol/chloroform protocol (<http://cshprotocols.cshlp.org/content/2010/6/pdb.prot5438.full>), ethanol precipitation protocol (<http://cshprotocols.cshlp.org/content/2010/6/pdb.prot5440.full>)

Citation: Daniel Richter RNA clean-up by phenol:chloroform. **protocols.io**

[dx.doi.org/10.17504/protocols.io.iv4ce8w](https://doi.org/10.17504/protocols.io.iv4ce8w)

Published: 04 Dec 2017

Protocol

Step 1.

Add water to bring volume to 400µL.

 **AMOUNT**

400 µl Additional info: volume

Step 2.

Add 40 µL (1/10 of final volume) of 3 M sodium acetate, pH 5.2.

 **AMOUNT**

40 µl Additional info: 3 M sodium acetate, pH 5.2

 **NOTES**

Ashley Humphrey 16 Aug 2017

Final concentration will be 0.3 M.

Step 3.

Add 880 µL (2x volume) of phenol:chloroform:isoamyl alcohol pH 8.0

 **AMOUNT**

880 µl Additional info:

Step 4.

Shake vigorously inside fume hood for 15 seconds.

Step 5.

Centrifuge at maximum speed in a microcentrifuge at 4° C, for 2 minutes.

Step 6.

Check solution for insoluble precipitate, indicating the presence of potassium salts > 20 mM.

Step 7.

Transfer 420 µL of the top aqueous phase to a new tube.

AMOUNT

420 µl Additional info: Transferred into a new tube.

Step 8.

Add 840 µL of chloroform:isoamyl alcohol pH 8.0

Step 9.

Shake vigorously inside fume hood for 15 seconds.

Step 10.

Centrifuge at max speed for 2 minutes at 4°C.

Step 11.

Transfer 400 µL of the top aqueous phase to a new tube.

Step 12.

Add 1.5 µL of GlycoBlue.

AMOUNT

1.5 µl Additional info: GlycoBlue

NOTES

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Final amount ~ 25 micrograms, working stock 15 micrograms/microliter.

Step 13.

Add 100 µl (2.5 volumes) of 100% ethanol

AMOUNT

100 µl Additional info:

ANNOTATIONS

Zachary Lewis 24 May 2018

Should be 1000 ul, right?

Step 14.

Shake vigorously inside fume hood for 15 seconds.

Step 15.

Freeze overnight at -20°C.

Step 16.

Centrifuge at maximum speed for 10 minutes at 4°C.

Step 17.

Pipette out ethanol, slowly, with P1000 then P10.

NOTES

Ashley Humphrey 16 Aug 2017

Avoid leaving liquid on sides of the tube.

Step 18.

Wash with -20 °C, 70% ethanol and centrifuge at maximum speed for 5 minutes at 4° C.

Step 19.

Pipette out ethanol, slowly, with P1000, then P10.

NOTES

Ashley Humphrey 16 Aug 2017

Avoid leaving liquid on sides of tube.

Step 20.

Dry pellet and resuspend in 27.5 µL of water.

AMOUNT

27.5 µl Additional info: Water

Step 21.

Spec on NanoDrop and dilute 1 µL aliquot of RNA to 1 ng/µL in a separate tube (for Bioanalyzer)

Warnings

Perform all phenol and chloroform procedures in the vent hood.