RNAqueous with No DNAse, DNA Removal by Acid Phenol:Chloroform

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Abstract

March, 2012, based on RNAqueous May 29, 2008 protocol revision C, 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: Dan Richter RNAqueous with No DNAse, DNA Removal by Acid Phenol:Chloroform. **protocols.io** dx.doi.org/10.17504/protocols.io.itbcein
Published: 04 Dec 2017

Guidelines

To avoid possible RNA degradation, try to work quickly in all steps.

Protocol

Elution Solution Preparation

Step 1.

Place elution solution in a dry bath at 70-80ºC

AMOUNT
60 µl Additional info: per pellet

Sample Lysate Preparation

Step 2.

Place pellet in a 50 mL conical tube on ice

Sample Lysate Preparation

Step 3.

Add lysis buffer

AMOUNT
700 µl Additional info:

Sample Lysate Preparation

Step 4.

Pipette up and down 5 times to resuspend pellet

Sample Lysate Preparation

Step 5.

On ice, add more lysis buffer

AMOUNT

700 µl Additional info:

Sample Lysate Preparation

Step 6.

Pipette up and down 20 times to fully resuspend/lyse pellet

Sample Lysate Preparation

Step 7.

Transfer into two 1.5 mL Eppendorf tubes on ice

AMOUNT

700 µl Additional info: each

Sample Lysate Preparation

Step 8.

Take an aliquot from one Eppendorf tube and pipette onto a glass slide to check lysis

AMOUNT

10 µl Additional info: aliquot

NOTES

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Keep the slide inside a petri dish to ensure the noxious smell is contained

Sample Lysate Preparation

Step 9.

Immediately centrifuge both tubes at max speed in a microcentrifuge at 1ºC for 5 min to pellet unlysed bacteria

Sample Lysate Preparation

Step 10.

During centrifugation, add cover slip to the glass slide and quickly confirm lysis using a microscope.

Sample Lysate Preparation

Step 11.

If cells were lysed, transfer supernatant into two new 1.5 mL Eppendorf tubes. Otherwise, continue with the same tubes.

Sample Lysate Preparation

Step 12.

On ice, pass the lysate in each tube 5 times through a 25 gauge needle fitted to an RNAse-free 1 mL syringe.

NOTES

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If the cells were not lysed, pass through the needle additional times, and re-check lysis before continuing.

Filter Binding, Washing and Elution of RNA

Step 13.

Add 64% EtOH at room temperature (RT) per tube

AMOUNT

700 µl Additional info:

Filter Binding, Washing and Elution of RNA

Step 14.

Mix thoroughly by inverting the tubes several times

Filter Binding, Washing and Elution of RNA

Step 15.

Transfer lysate/ethanol mixture from one of the Eppendorf tubes to a single filter cartridge in a collection tube

AMOUNT

700 µl Additional info:

Filter Binding, Washing and Elution of RNA

Step 16.

Centrifuge at 15,000 xg, RT, 30 sec or until the mixture is drawn through the filter

Filter Binding, Washing and Elution of RNA

Step 17.

Discard the flow through

Filter Binding, Washing and Elution of RNA

Step 18.

Repeat steps 15-17 three additional times until all of the lysate/ethanol mixture in both Eppendorf tubes has been drawn through the filter cartridge

Filter Binding, Washing and Elution of RNA

Step 19.

Add wash solution #1

AMOUNT
700 µl Additional info:

Filter Binding, Washing and Elution of RNA
Step 20.

Centrifuge at 15,000 xg, RT, 30 sec

Filter Binding, Washing and Elution of RNA
Step 21.

Discard the flow through

Filter Binding, Washing and Elution of RNA
Step 22.

Add wash solution #2/3

AMOUNT
500 µl Additional info:

Filter Binding, Washing and Elution of RNA
Step 23.

Centrifuge at 15,000 xg, RT, 30 sec

Filter Binding, Washing and Elution of RNA
Step 24.

Discard the flow through

Filter Binding, Washing and Elution of RNA
Step 25.

Repeat steps 22-24

Filter Binding, Washing and Elution of RNA
Step 26.

Centrifuge an additional 30 sec at 15,000 x g to remove residual wash solution #2/3

Filter Binding, Washing and Elution of RNA
Step 27.

Place filter cartridge in a new collection tube

Filter Binding, Washing and Elution of RNA
Step 28.

Pipette pre-heated elution solution onto the filter

AMOUNT
40 µl Additional info:

Filter Binding, Washing and Elution of RNA

Step 29.

Centrifuge at 15,000 x g, 30 sec

Filter Binding, Washing and Elution of RNA

Step 30.

Pipette additional pre-heated elution solution onto the filter

AMOUNT
10 µl Additional info:

Filter Binding, Washing and Elution of RNA

Step 31.

Centrifuge at 15,000 x g, 30 sec

NOTES
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The final volume of eluate should be ~45 uL

Filter Binding, Washing and Elution of RNA

Step 32.

Spec on NanoDrop and make a dilution at 1:5 (2 µl :8 µl) in a separate tube for PCR/Bioanalyzer

AMOUNT
2 µl Additional info: aliquot

Filter Binding, Washing and Elution of RNA

Step 33.

Take an aliquot of 1:5 dilution from previous step and dilute to 1 ng/µL in a seperate tube (for Bioanalyzer)

AMOUNT
1 µl Additional info: aliquot

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 34.

Add water to bring volume to 400 µL

AMOUNT
358.5 µl Additional info: water

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 35.

Add 3 M sodium acetate at pH = 5.2 (final concentration = 0.3 M)

AMOUNT
40 µl Additional info: (1/10 volume)

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 36.

Add acid phenol:chloroform:isoamyl alcohol at pH = 4.5

AMOUNT
880 µl Additional info: 2 volumes

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 37.

Shake vigorously inside the fume hood for 15 sec

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 38.

Centrifuge at max speed in a microcentrifuge at 4ºC for 2 min

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

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 39.

Transfer the top/aqueous phase to a new tube

AMOUNT
430 µl Additional info:

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 40.

Add acid phenol:chloroform:isoamyl alcohol at pH = 4.5

AMOUNT
860 µl Additional info: (2 volumes)

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 41.

Shake vigorously inside fume hood for 15 sec

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 42.

Centrifuge at max speed in a microcentrifuge at 4ºC for 2 min

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 43.

Transfer the top/aqueous phase to a new tube

AMOUNT

420 µl Additional info:

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 44.

Add acid phenol:chloroform:isoamyl alcohol at pH = 4.5

AMOUNT

840 µl Additional info: (2 volumes)

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 45.

Shake vigorously inside fume hood for 15 sec

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 46.

Centrifuge at max speed in a microcentrifuge at 4ºC for 2 min

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 47.

Transfer the top/aqueous phase to a new tube

AMOUNT

410 µl Additional info:

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 48.

Add chlorofom:isoamyl alcohol

AMOUNT

820 µl Additional info: (2 volumes)

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 49.

Shake vigorously inside fume hood for 15 sec

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 50.

Centrifuge at max speed in a microcentrifuge at 4ºC for 2 min

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 51.

Transfer the top/aqueous phase to a new tube

AMOUNT

400 µl Additional info:

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 52.

Add GlycoBlue (final amount 25 µg, working stock 15 µg/µL)

AMOUNT

1.5 µl Additional info:

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 53.

Add 100% EtOH

AMOUNT

1 ml Additional info: (2.5 volumes)

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 54.

Shake vigorously to mix

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 55.

Freeze overnight at -20ºC

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 56.

Centrifuge at max speed in a microcentrifuge for 10 min at 4ºC

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 57.

Decant ethanol then remove residual with pipette

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 58.

Wash with -20ºC 70% ethanol

AMOUNT

1 ml Additional info:

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 59.

Centrifuge for 5 min at 4ºC

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 60.

Dry pellet and resuspend in water

AMOUNT
27.5 µl Additional info:

Triple Acid Phenol:Chloroform Extraction to Remove DNA

Step 61.

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

AMOUNT
1 µl Additional info: aliquot