RNAqueous with DNAse Clean-up by Phenol:Chloroform

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Abstract

February, 2012, based on RNAqueous May 29, 2008 protocol revision C, TURBO DNA-free June 9, 2009 protocol 1907M revision F, 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)

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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 the 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

AMOUNT

700 µl Additional info: each

Sample Lysate Preparation

Step 8.

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

AMOUNT

10 µl Additional info:

NOTES

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

Sample Lysate Preparation

Step 9.

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

Sample Lysate Preparation

Step 10.

During centrifugation, add a 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 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 drawm 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 once

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 the 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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Final volume of eluate should be ~45 uL

TURBO DNAse Treatment

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

TURBO DNAse Treatment

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

NOTES
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If the concentration is >240 ng/uL (10ug/41.5 uL), dilute an aliquot to 240 ng/uL in 41.5 uL and save remainder at -80C.

TURBO DNAse Treatment

Step 34.

Add SUPERase-In (at 20 U / µl, final concentration 1 U / µl)

AMOUNT
2.5 µl Additional info:

TURBO DNAse Treatment

Step 35.

Add 10X TURBO DNAse buffer

AMOUNT
5 µl Additional info:

TURBO DNAse Treatment

Step 36.

Add TURBO DNAse and mix gently

AMOUNT
1 µl Additional info:

TURBO DNAse Treatment

Step 37.

Incubate at 37ºC for 30 min

Phenol:Chloroform Extraction

Step 38.

Add water to bring volume to 400 µL

AMOUNT
350 µl Additional info: water

Phenol:Chloroform Extraction

Step 39.

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

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

Phenol:Chloroform Extraction

Step 40.

Add phenol:chloroform:isoamyl alcohol at pH = 8

AMOUNT
880 µl Additional info: (2 volumes)

Phenol:Chloroform Extraction

Step 41.

Shake vigorously inside fume hood for 15 sec

Phenol:Chloroform Extraction

Step 42.

Centrifuge at max speed in a microcentrifuge for 2 min

NOTES

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

Phenol:Chloroform Extraction

Step 43.

Transfer the top/aqueous phase to a new tube

AMOUNT

420 µl Additional info:

Phenol:Chloroform Extraction

Step 44.

Add chloroform:isoamyl alcohol at pH = 8

AMOUNT

840 µl Additional info: (2 volumes)

Phenol:Chloroform Extraction

Step 45.

Shake vigorously inside fume hood for 15 sec

Phenol:Chloroform Extraction

Step 46.

Centrifuge at max speed in a microcentrifuge for 2 min

Phenol:Chloroform Extraction

Step 47.

Transfer the top/aqueous phase to a new tube

AMOUNT

400 µl Additional info:

Phenol:Chloroform Extraction

Step 48.

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

AMOUNT

1.5 µl Additional info:

Phenol:Chloroform Extraction

Step 49.

Add 100% EtOH

AMOUNT
1000 µl Additional info: (2.5 volumes)

Phenol:Chloroform Extraction
Step 50.

Shake vigorously to mix

Phenol:Chloroform Extraction
Step 51.

Freeze overnight at -20ºC

Phenol:Chloroform Extraction
Step 52.

Thaw

Phenol:Chloroform Extraction
Step 53.

Centrifuge at max speed in a microcentrifuge for 10 min

Phenol:Chloroform Extraction
Step 54.

Decant ethanol, then remove residual with pipette.

Phenol:Chloroform Extraction
Step 55.

Wash with -20ºC 70% EtOH

Phenol:Chloroform Extraction
Step 56.

Centrifuge for 5 min, 4ºC

Phenol:Chloroform Extraction
Step 57.

Dry pellet and resuspend in water

AMOUNT
50 µl Additional info:

Phenol:Chloroform Extraction
Step 58.

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

AMOUNT

1 µl Additional info: aliquot