# RNAqueous with TURBO DNA-free and SUPERase-In

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## Abstract

Daniel Richter, Nov 29, 2011

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

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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 pellet

## Sample Lysate Preparation

**Step 5.**

While still on ice, add additional 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.**

Aliquot onto glass slide to check lysis from one Eppendorf tube

AMOUNT
10 µl Additional info:

NOTES
**Alyssa Alsante** 01 Jul 2017

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, 5 min to pellet unlysed bacteria

## Sample Lysate Preparation

**Step 10.**

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

NOTES
**Alyssa Alsante** 01 Jul 2017

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

## Sample Lysate Preparation

**Step 11.**

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

NOTES
**Alyssa Alsante** 01 Jul 2017

If the cells were not lysed in step 2, pass through the needle an additional time, and re-check lysis before continuing.

Filter Binding, Washing and Elution of RNA
**Step 12.**

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

AMOUNT
700 µl Additional info:

Filter Binding, Washing and Elution of RNA
**Step 13.**

Mix thoroughly by inverting the tubes several times

Filter Binding, Washing and Elution of RNA
**Step 14.**

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 15.**

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 16.**

Discard the flow through

Filter Binding, Washing and Elution of RNA
**Step 17.**

Repeat steps 14-16 three additional times until all of the lysate/ethanol mixture in both tubes has been drawn through the filter cartridge

Filter Binding, Washing and Elution of RNA
**Step 18.**

Add wash solution #1

AMOUNT
700 µl Additional info:

Filter Binding, Washing and Elution of RNA
**Step 19.**

Centrifuge at 15,000 xg, RT, 30 sec

Filter Binding, Washing and Elution of RNA
**Step 20.**

Discard the flow through

Filter Binding, Washing and Elution of RNA
**Step 21.**

Add wash solution #2/3

AMOUNT
500 µl Additional info:

Filter Binding, Washing and Elution of RNA
**Step 22.**

Centrifuge at 15,000 x g, RT, 30 sec

Filter Binding, Washing and Elution of RNA
**Step 23.**

Discard the flow through

Filter Binding, Washing and Elution of RNA
**Step 24.**

Repeat wash steps 21-23

Filter Binding, Washing and Elution of RNA
**Step 25.**

Centrifuge an additional 30 sec at 15,000 x g

NOTES
**Alyssa Alsante** 03 Jul 2017

This step removes residual wash solution #2/3

Filter Binding, Washing and Elution of RNA
**Step 26.**

Place filter cartridge in a new collection tube

Filter Binding, Washing and Elution of RNA

**Step 27.**

Pipette pre-heated elution solution onto the filter

AMOUNT

40 µl Additional info:

Filter Binding, Washing and Elution of RNA

**Step 28.**

Centrifuge at 15,000 xg, 30 sec

Filter Binding, Washing and Elution of RNA

**Step 29.**

Pipette an additional pre-heated elution solution onto the filter

AMOUNT

10 µl Additional info:

Filter Binding, Washing and Elution of RNA

**Step 30.**

Centrifuge at 15,000 x g, 30 sec

NOTES

**Alyssa Alsante** 03 Jul 2017

Final volume of eluate should be ~45 uL

TURBO DNAse Treatment

**Step 31.**

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 32.**

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

NOTES

**Alyssa Alsante** 03 Jul 2017

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

TURBO DNAse Treatment

**Step 33.**

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

AMOUNT

2.5 µl Additional info:

TURBO DNAse Treatment

**Step 34.**

Add 10X TURBO DNAse Buffer

AMOUNT

1 µl Additional info:

TURBO DNAse Treatment

**Step 35.**

Incubate at 37ºC for 30 min

TURBO DNAse Treatment

**Step 36.**

Vortex DNAse Inactivation Reagent to resuspend

TURBO DNAse Treatment

**Step 37.**

Add DNAse Inactivation Reagent

AMOUNT

5 µl Additional info:

TURBO DNAse Treatment

**Step 38.**

Incubate 5 min at 22-26ºC, flicking to mix 2-3 times during incubation.

TURBO DNAse Treatment

**Step 39.**

Centrifuge at max speed for 1.5 min at RT

TURBO DNAse Treatment

**Step 40.**

Transfer RNA to a fresh tube

AMOUNT

50 µl Additional info:

NOTES

**Alyssa Alsante** 03 Jul 2017

To avoid transferring beads, pipette out at an angle and rotate tube such that beads are on the bottom.

## TURBO DNAse Treatment

**Step 41.**

Repeat centrifugation/transfer (steps 39-40) 2-3 times until no beads are transferred

## TURBO DNAse Treatment

**Step 42.**

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