

Version 2 ▼

© Purification of RNA from a DNA/RNA Extract V.2

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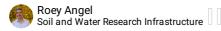
¹Soil and Water Research Infrastructure

1 Works for me

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ABSTRACT

The following protocol is intended as a downstream application for our <u>Total Nucleic Acids Extraction from Soil</u> protocol. This protocol describes how to purify RNA from a DNA and RNA extract using <u>TURBO™ DNase</u> and <u>GeneJET RNA Cleanup and Concentration Micro Kit</u>. This protocol is a simplified and condensed version of the full protocols provided by the manufacturers.

ATTACHMENTS

4393900B.pdf MAN0012671_GeneJET_ RNA_Cleanup_Concentrati on Micro UG.pdf THE RNA storage solution.pdf

MATERIALS

NAME	CATALOG #	VENDOR
TURBO™ DNase (2 U/µL)	AM2238	Thermo Fisher Scientific
GeneJET RNA Cleanup and Concentration Micro Kit	K0841	Thermo Fisher Scientific
Ethanol, Absolute, Molecular Biology Grade	BP2818500	Thermo Fisher Scientific
RNase AWAY™ Surface Decontaminant	A998.4	Carl Roth
THE RNA Storage Solution	AM7000	Thermo Fisher Scientific
RNaseOUT Recombinant Ribonuclease Inhibitor	10777019	Thermo Fisher Scientific

STEPS MATERIALS

NAME	CATALOG #	VENDOR
TURBO™ DNase (2 U/µL)	AM2238	Thermo Fisher Scientific
RNaseOUT™ Recombinant Ribonuclease Inhibitor	10777019	Thermo Fisher Scientific
USB Dithiothreitol (DTT) 0.1M Solution	707265ML	Thermo Fisher Scientific
Nuclease-free autoclaved DEPC-treated water	T143.1	Carl Roth
GeneJET RNA Cleanup and Concentration Micro Kit	K0841	Thermo Fisher Scientific
Ethanol, Absolute, Molecular Biology Grade	BP2818500	Thermo Fisher Scientific
THE RNA Storage Solution	AM7000	Thermo Fisher Scientific

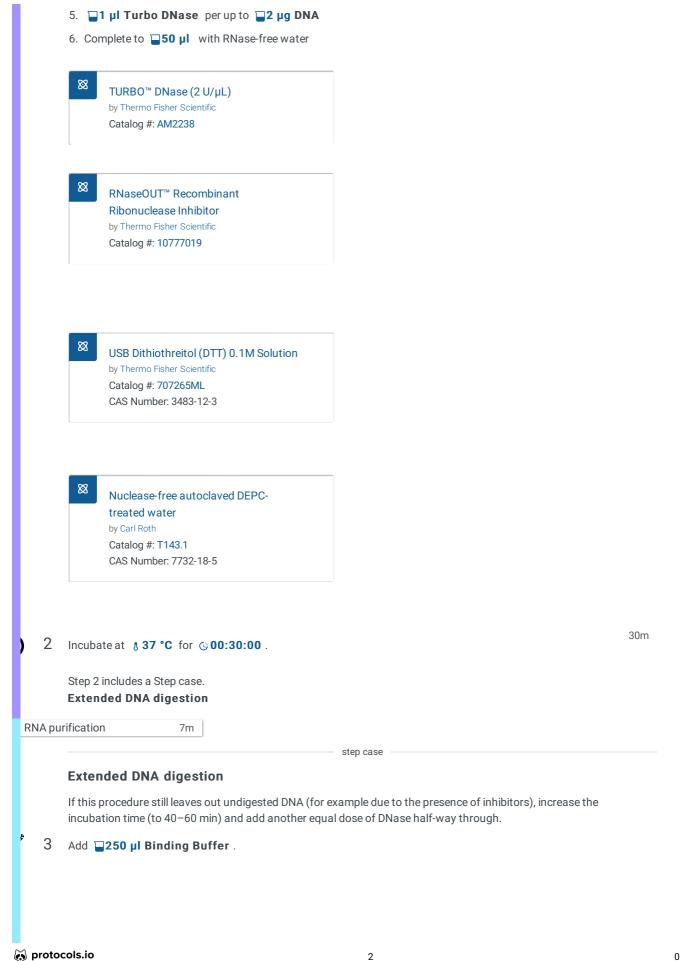
BEFORE STARTING

- 1. For each sample prepare one Gene JET RNA Purification Micro Column tube and two RNase–free collection tubes (1.5 ml).
- 2. Add the required amount of ethanol to Wash Buffer 1 and Wash Buffer 2 (amount is dependent on kit size).

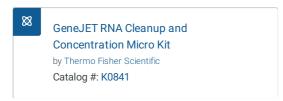
DNA digestion 45m

- 1 Prepare the following mixture in a 1.5 ml tube:
 - 1. $\square 10 \ \mu I$ to $\square 42 \ \mu I$ of TNA extract ($\square 1 \ \mu g$ to $\square 3 \ \mu g$ of DNA).
 - 2. To pl TURBO DNase buffer 10x
 - 3. **1 µl RNaseOUT**
 - 4. **□1 μl 0,1M DTT**

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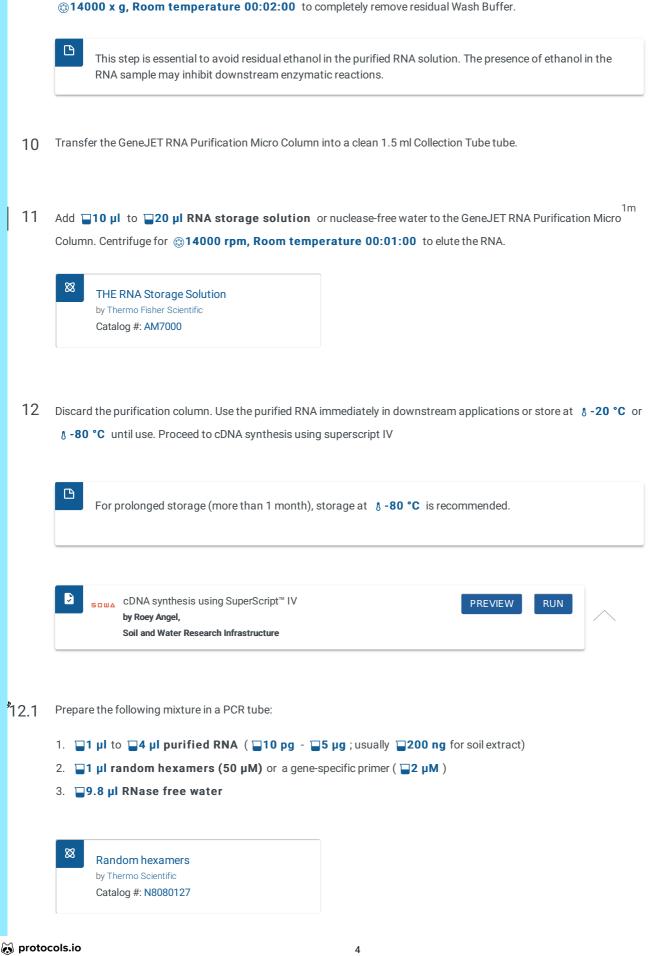


4 Add **□300 µl absolute ethanol**.

Ethanol, Absolute, Molecular Biology
Grade
by Thermo Fisher Scientific
Catalog #: BP2818500

- Transfer the mixture to the Gene JET RNA Purification Micro Column preassembled with a collection tube. Centrifuge the column for **314000 x g, Room temperature 00:01:00**. Discard the flow-through. Place the Gene JET RNA Purification Micro Column back into the collection tube.
- 6 Add **3700 μl Wash Buffer 1** (supplemented with ethanol) to the GeneJET RNA Purification Micro Column and centrifuge for **314000 x g, Room temperature 00:01:00**. Discard the flow-through and place the purification column back into the collection tube.
 - GeneJET RNA Cleanup and
 Concentration Micro Kit
 by Thermo Fisher Scientific
 Catalog #: K0841
- 7 Add 700 µl Wash Buffer 2 (supplemented with ethanol) to the GeneJET RNA Purification Micro Column and centrifuge for 14000 x g, Room temperature 00:01:00. Discard the flow-through and place the purification column back into the collection tube.
 - GeneJET RNA Cleanup and
 Concentration Micro Kit
 by Thermo Fisher Scientific
 Catalog #: K0841
- 8 Repeat step 7.
- 9 Centrifuge the empty GeneJET RNA Purification Micro Column for an additional

2_m

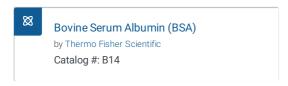


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- 12.2 Mix gently and spin down the solution.
- 12.3 Incubate the mixture at § 65 °C for © 00:05:00 in a thermocycler and chill § On ice (or in the cycler at > § 4 °C) for at least © 00:01:00.
- 12.4 Prepare the following mixture and add to each tube:
 - 1. **4 μl 5x Reaction buffer**
 - 2. **1 μl dNTP mix, 10 mM**
 - 3. **□1 μl 0.1M DTT**
 - 4. **□1 μl RNaseOUT™ (40 U/μl)** *
 - 5. **30.2 μl BSA (20 μg/μl)**
 - 6. □1 μl SuperScript™ IV RT (200 units/μl)
 - * Optional
 - USB Dithiothreitol (DTT) 0.1M Solution
 by Thermo Fisher Scientific
 Catalog #: 707265ML
 CAS Number: 3483-12-3
 - SuperScript™ IV First-Strand Synthesis
 System
 by Thermo Fisher Scientific
 Catalog #: 18091050
 - RNaseOUT™ Recombinant
 Ribonuclease Inhibitor
 by Thermo Fisher Scientific
 Catalog #: 107777019

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- Incubate the mixture in a thermocycler at § 23 °C for © 00:10:00 (only if using random hexamers, skip if using a specific primer) followed by § 50 °C for © 01:00:00 to © 03:00:00 and § 80 °C for © 00:10:00 . Chill § On ice .
-)12.6 For PCR templates > 1kb remove the RNA by adding $\Box 1 \mu I$ (2 units) of E. coli RNase H and incubate at @ 37 °C for @ 00:20:00 .
 - Ribonuclease H (RNase H)
 by Thermo Fisher Scientific
 Catalog #: 18021071
- 12.7 Prepare the following mixture and add to each tube:
 - 1. **□1 μl DNA Polymerase I reaction buffer**
 - 2. DNA Polymerase I
 - 3. **Q**0.2 μl RNase H
 - 4. 3.05 μl RNase-free water
 - 5. **3** μl Template cDNA
 - DNA Polymerase I (10 U/μL)
 by Thermo Fisher Scientific
 Catalog #: EP0041
 - Ribonuclease H (RNase H)
 by Thermo Fisher Scientific
 Catalog #: 18021071
 - Nuclease-free autoclaved DEPCtreated water
 by Carl Roth
 Catalog #: T143.1
 CAS Number: 7732-18-5

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12.8 Incubate for at § 15 °C for © 02:00:00 followed by © 00:10:00 at § 75 °C for deactivation.

3. Purify the reaction through phenol/chloroform purification followed by ethanol precipitation or using a PCR purification kit.