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

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Version 2
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1 Works for me
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SoWa RI Anaerobic and Molecular Microbiology (public)
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ABSTRACT

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

ATTACHMENTS

4393900B.pdf
MAN0012671_GeneJET_RNA_Cleanup_Concentration_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. **10 µl** to **42 µl** of TNA extract (**1 µg** to **3 µg** of DNA).
2. **5 µl TURBO DNase buffer 10x**
3. **1 µl RNaseOUT**
4. **1 µl 0,1M DTT**

Citation: Roey Angel, Eva Petrova (04/30/2020). Purification of RNA from a DNA/RNA Extract. https://dx.doi.org/10.17504/protocols.io.bftbjnin

5. **1 µl Turbo DNase** per up to **2 µg DNA**

6. Complete to **50 µl** with RNase-free water

TURBO™ DNase (2 U/µL)
by Thermo Fisher Scientific
Catalog #: AM2238

RNaseOUT™ Recombinant Ribonuclease Inhibitor
by Thermo Fisher Scientific
Catalog #: 10777019

USB Dithiothreitol (DTT) 0.1M Solution
by Thermo Fisher Scientific
Catalog #: 707265ML
CAS Number: 3483-12-3

Nuclease-free autoclaved DEPC-treated water
by Carl Roth
Catalog #: T143.1
CAS Number: 7732-18-5

2 Incubate at **37 °C** for **00:30:00** . 30m

Step 2 includes a Step case.
Extended DNA digestion

RNA purification 7m

step case

Extended DNA digestion

If this procedure still leaves out undigested DNA (for example due to the presence of inhibitors), increase the incubation time (to 40–60 min) and add another equal dose of DNase half-way through.

3 Add **250 µl Binding Buffer** .

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GeneJET RNA Cleanup and Concentration Micro Kit
by Thermo Fisher Scientific
Catalog #: K0841

4 Add **300 µl absolute ethanol** .

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

5 Transfer the mixture to the Gene JET RNA Purification Micro Column preassembled with a collection tube. Centrifuge the column for **14000 x g, Room temperature 00:01:00** . Discard the flow-through. Place the GeneJET RNA Purification Micro Column back into the collection tube. 1m

6 Add **700 µl Wash Buffer 1** (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. 1m

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. 1m

GeneJET RNA Cleanup and Concentration Micro Kit
by Thermo Fisher Scientific
Catalog #: K0841

8 Repeat step 7. 1m

9 Centrifuge the empty GeneJET RNA Purification Micro Column for an additional 2m

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14000 x g, Room temperature 00:02:00 to completely remove residual Wash Buffer.

This step is essential to avoid residual ethanol in the purified RNA solution. The presence of ethanol in the RNA sample may inhibit downstream enzymatic reactions.

10 Transfer the GeneJET RNA Purification Micro Column into a clean 1.5 ml Collection Tube tube.

11 Add **10 µl** to **20 µl RNA storage solution** or nuclease-free water to the GeneJET RNA Purification Micro Column. Centrifuge for **14000 rpm, Room temperature 00:01:00** to elute the RNA. 1m

THE RNA Storage Solution
by Thermo Fisher Scientific
Catalog #: AM7000

12 Discard the purification column. Use the purified RNA immediately in downstream applications or store at **-20 °C** or **-80 °C** until use. Proceed to cDNA synthesis using superscript IV

For prolonged storage (more than 1 month), storage at **-80 °C** is recommended.

cDNA synthesis using SuperScript™ IV
by Roey Angel,
Soil and Water Research Infrastructure
PREVIEW RUN

12.1 Prepare the following mixture in a PCR tube:

1. **1 µl** to **4 µl purified RNA** (**10 pg** - **5 µg** ; usually **200 ng** for soil extract)
2. **1 µl random hexamers (50 µM)** or a gene-specific primer (**2 µM**)
3. **9.8 µl RNase free water**

Random hexamers
by Thermo Scientific
Catalog #: N8080127

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Nuclease-free autoclaved DEPC-treated water
by Carl Roth
Catalog #: T143.1
CAS Number: 7732-18-5

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. **0.2 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 #: 10777019

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Bovine Serum Albumin (BSA)
by Thermo Fisher Scientific
Catalog #: B14

12.5 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 **1 µl** (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. **0.75 µl DNA Polymerase I**
3. **0.2 µl RNase H**
4. **3.05 µl RNase-free water**
5. **5 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 DEPC-treated 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.

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

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