# Purification of RNA from a DNA/RNA Extract V.1

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Version 1
Apr 30, 2020
1 Works for me
dx.doi.org/10.17504/protocols.io.qdqds5w

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 |
|---|---|---|
| USB Dithiothreitol (DTT) 0.1M Solution | 707265ML | Thermo Fisher Scientific |
| RNaseOUT™ Recombinant Ribonuclease Inhibitor | 10777019 | Thermo Fisher Scientific |
| TURBO™ DNase (2 U/µL) | AM2238 | 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.qdqds5w

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 digest**

RNA purification 7m

step case

## Extended digest

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
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8 Repeat step 7. 1m
**go to step #7 undefined**

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.

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



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