VERSION 2

JAN 22, 2021

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.brqum5ww

Protocol Citation: Roey Angel, Eva Petrova 2021. cDNA synthesis using SuperScript™ IV. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.brqum5ww> Version created by Roey Angel

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Jan 22, 2021

Last Modified: Jan 22, 2021

PROTOCOL integer ID:
 46580

Keywords: cDNA, RNA, mRNA, SuperScript, reverse transcriptase

cDNA synthesis using SuperScript™ IV V.2

In 1 collection

Roey Angel¹, Eva Petrova¹

¹Soil and Water Research Infrastructure

SoWa RI Anaerobic and Molecular Microbiology (public)

Tech. support email: eva.petrova@bc.cas.cz

Roey Angel

Soil and Water Research Infrastructure

ABSTRACT

The following protocol is intended as a downstream application for our [Purification of RNA from a DNA/RNA Extract](#) protocol. This protocol describes how to synthesise a first-strand non-specific complementary DNA (cDNA) from a purified RNA extract using [SuperScript IV Reverse Transcriptase](#). The second strand synthesis is usually not required for most downstream applications. This protocol is a simplified and condensed version of the full protocol provided by the manufacturer.

ATTACHMENTS

[SSIV_First_Strand_Synthesis_System_UG.pdf](#)

MATERIALS

MATERIALS

☒ Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #B14**

☒ SuperScript™ IV First-Strand Synthesis System **Thermo Fisher Scientific Catalog #18091050**

☒ RNaseOUT™ Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #10777019**

☒ Ribonuclease H (RNase H) **Thermo Fisher Scientific Catalog #18021071**

☒ DNA Polymerase I (10 U/μL) **Thermo Fisher Scientific Catalog #EP0041**

☒ Random hexamers **Thermo Scientific Catalog #N8080127**

STEP MATERIALS

☒ Random hexamers **Thermo Scientific Catalog #N8080127**

☒ Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #T143.1**

☒ USB Dithiothreitol (DTT) 0.1M Solution **Thermo Fisher Scientific Catalog #707265ML**

☒ RNaseOUT™ Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #10777019**

☒ SuperScript™ IV First-Strand Synthesis System **Thermo Fisher Scientific Catalog #18091050**

☒ Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #B14**

☒ Ribonuclease H (RNase H) **Thermo Fisher Scientific Catalog #18021071**

☒ DNA Polymerase I (10 U/μL) **Thermo Fisher Scientific Catalog #EP0041**

☒ Ribonuclease H (RNase H) **Thermo Fisher Scientific Catalog #18021071**

☒ Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #T143.1**

BEFORE START INSTRUCTIONS

Make sure your RNA is pure and contains no traces of DNA. A simple and very sensitive way to ensure that is to use the purified RNA as a template for a PCR reaction targeting a gene that should be present in the sample. A negative result indicates a lack of DNA template in the sample.

Primer annealing

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 **Thermo Scientific Catalog #N8080127**

Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #T143.1**

2 Mix gently and spin down the solution.

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 .

Reverse transcription

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 **Thermo Fisher Scientific Catalog #707265ML**

SuperScript™ IV First-Strand Synthesis System **Thermo Fisher Scientific Catalog #18091050**

RNaseOUT™ Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #10777019**

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

dNTP Mix (10 mM each) **Thermo Fisher Scientific Catalog #R0191**

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 .

- 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) **Thermo Fisher Scientific Catalog #18021071**

Optional: Second strand synthesis

- 7 Prepare the following mixture and add to each tube:

- 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) **Thermo Fisher Scientific Catalog #EP0041**

Ribonuclease H (RNase H) **Thermo Fisher Scientific Catalog #18021071**

Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #T143.1**

- 8 Incubate for at 15 °C for 02:00:00 followed by 00:10:00 at 75 °C for deactivation.

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