Version 2

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cDNA synthesis using SuperScript™ IV V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.brqum5ww

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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

DOI

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PROTOCOL CITATION

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cDNA, RNA, mRNA, SuperScript, reverse transcriptase

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MATERIALS TEXT

MATERIALS

Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #B14** Step 4

SuperScript™ IV First-Strand Synthesis System **Thermo Fisher Scientific Catalog #18091050** Step 4

RNaseOUT™ Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #10777019** Step 4

Ribonuclease H (RNase H) **Thermo Fisher Scientific Catalog #18021071** In 2 steps

DNA Polymerase I (10 U/µL) **Thermo Fisher Scientific Catalog #EP0041** Step 7

Random hexamers **Thermo Scientific Catalog #N8080127** Step 1

STEP MATERIALS

Random hexamers **Thermo Scientific Catalog #N8080127** Step 1

Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #T143.1** In 2 steps

USB Dithiothreitol (DTT) 0.1M Solution **Thermo Fisher Scientific Catalog #707265ML** Step 4

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Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #T143.1** In 2 steps

BEFORE STARTING

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

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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**

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

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

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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. **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.

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