

TruScript First Strand cDNA Synthesis Kit for mRNA
Product # 54400

Product Insert

Norgen's TruScript First Strand cDNA Synthesis Kit for mRNA is an all-in-one, ready-to-use product for simple set up of reverse transcription of messenger RNA (poly A-containing transcripts). The kit contains the 2x Reaction Mix and the TruScript Enzyme Mix. The 2x Reaction Mix contains a blend of buffer, nucleotides and primers for effective cDNA synthesis from total RNA transcripts or enriched mRNA samples.

The kit utilizes Norgen's TruScript Reverse Transcriptase, a mutant version of Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase. It has reduced RNase H activity and increased thermal stability. Norgen's TruScript Reverse Transcriptase is of a high purity and thus could function in cDNA synthesis with high thermal stability, high fidelity, and high specificity. The TruScript Reverse Transcriptase has a broad range of working temperatures from 37°C to 60°C, with cDNA product sizes from 100 bp to 12 kb.

Kit Components

Component	Product # 54400 (50 Reactions)
TruScript Enzyme Mix	100 µL
2x Reaction Mix for mRNA	500 µL
Nuclease-Free Water	1.25 mL
Product Insert	1

Storage Conditions and Product Stability

Norgen's TruScript First Strand cDNA Synthesis Kit for mRNA components should be stored at -20°C. These reagents should remain stable for at least 1 year in their unopened containers.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

Procedure for First-Strand cDNA Synthesis

Materials to be supplied by user

- Thermocycler

1. The procedure can be used for 1 pg to 1 µg of total RNA (or 1 pg to 500 ng of mRNA). It is highly recommended that RNA of a high quality (such as those isolated with Norgen's RNA purification products) be used.

Note: Higher amounts (>1 µg) of RNA input could be used. However, it is highly recommended that the volume of the reaction be scaled up.

2. Set up the First-Strand cDNA Synthesis reaction in a tube compatible with the thermocycler to be used, as described in **Table 1**.

Table 1. First-Strand cDNA Synthesis Reaction Set-up

Components	Volume per Reaction
2x Reaction Mix for mRNA	10 μ L
TruScript Enzyme Mix	2 μ L
RNA template (1 pg to 1 μ g total RNA)	x μ L
Nuclease-Free Water	x μ L
Total Volume	20 μL

3. Incubate First-Strand cDNA Synthesis reaction in a thermocycler as described in **Table 2**.

Table 2. Reaction Protocol First-Strand cDNA Synthesis Reaction

Temperature	Time
50°C ¹	30 - 60 minutes
70°C	15 minutes
4°C	Hold

¹ The suggested 50°C incubation temperature could be increased to 55°C for difficult templates (such as templates with high degree of secondary structure).

4. The cDNA generated can now be used as a template in a PCR reaction. In general, use 1 - 5 μ L of the cDNA in a 20 μ L PCR reaction (such as with Norgen's 2x PCR Master Mix, Cat.# 28007). Un-used cDNA should be stored at -20°C

Note: For some cDNA generated, it may be necessary to remove the RNA complementary to the cDNA prior to PCR amplification. This is particularly applicable to PCR targets of 1 kb or larger. Add 2 units of RNase H to the cDNA generated and incubate at 37°C for 20 minutes.

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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