NORGEN BIOTEK CORP.

3430 Schmon Pkwy, Thorold, ON, Canada L2V 4Y6
Phone: 1-866-667-4362 • 905-227-8848
Fax: 905-227-1061
Email: techsupport@norgenbiotek.com

TruScript™ Reverse Transcriptase Product Insert

Product # 54440

Kit Components

Component	Product # 54440 (10,000 units)
TruScript™ Reverse Transcriptase (200 units / µL)	50 µL
5x RT Buffer	1 mL
Nuclease-Free Water	1.25 mL
Product Insert	1

Norgen's TruScript™ Reverse Transcriptase is a mutant version of Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase. It has reduced RNase H activity and increased thermal stability. The TruScript™ Reverse Transcriptase has a broad range of working temperatures from 37°C to 60°C, with cDNA product sizes up to 12 kb.

Storage Conditions and Product Stability
Norgen's TruScript™ Reverse Transcriptase and the 5x RT Buffer 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.

Unit Definition
One unit of the enzyme incorporates 1 nmole of dTTP into acid-precipitable material in 10 minutes at 37°C using poly (A):oligo $(dT)_{25}$ as template-primer.

Procedure for First-Strand cDNA Synthesis

Materials to be supplied by user
- 10 mM dNTP Mix (Norgen Cat.# 28106)
- RNase Inhibitor (Optional)
- Thermocycler

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

   Note: A higher amount (>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
5x RT Buffer	4 µL
10 mM dNTP mix	1 µL
Reverse Transcription Primer[1]	1 µL
RNase Inhibitor (Optional)[2]	25 units
TruScript™ Reverse Transcriptase (200 units / µL)	1 µL
RNA template (1 pg to 1 µg total RNA)[3]	x µL
Nuclease-Free Water	x µL
Total Volume	**20 µL**

[1] Reverse Transcription Primer stock could be 50 µM (50 pmol / µL) of oligo $(dT)_{20}$; or 200 - 500 ng / µL of random primer; or 2 µM (2 pmol / µL) of gene-specific primer.
[2] The use of RNase Inhibitor is optional. It is highly recommended for use with RNA templates of 50 ng or less.
[3] RNA template could be added to the reaction as indicated. Alternatively, the RNA could be premixed with Reverse Transcription Primer and Nuclease-Free Water. This mix could then be heated at 65°C for 5 minutes and incubated on ice, prior to adding the rest of the reaction components. This is particularly beneficial for RNA templates known to have a high degree of secondary structure.

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
25°C (Optional)[1]	5 minutes
50°C[2]	30-60 minutes
70°C	15 minutes
4°C	Hold

[1] The 25°C incubation should be added when random primers are used in the reaction
[2] The suggested 50°C incubation temperature could be increased to 55°C for difficult templates (such as templates with a 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 µL of the cDNA in a 20 µL 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.