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# ValueScript Reverse Transcriptase — Product Insert

**Product # 54460**

**Kit Components**

| Component | Product # 54460 (10,000 units) |
|---|---|
| ValueScript Reverse Transcriptase (200 units / µL) | 50 µL |
| 5x RT Buffer | 1 mL |
| Nuclease-Free Water | 1.25 mL |
| Product Insert | 1 |

Norgen's ValueScript 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 ValueScript Reverse Transcriptase has an optimal working temperature of 42°C, with cDNA product sizes up to 12 kb.

**Storage Conditions and Product Stability**
Norgen's ValueScript 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. In a sterile RNase-free PCR tube compatible with the thermocycler to be used, add 1 µL of primers (200-500 ng of oligo $(dT)_{12-18}$, 50-250 ng of random primer or 2 pmol of specific primers) to

up to 10 µL of the RNA template. Heat the tube to 70°C for 5 minutes and incubate on ice (or 4°C on the thermocycler) for 1 minute to denature any possible secondary structure within the template. Spin briefly to collect the solution at the bottom of the tube.

3. 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 |
| RNase Inhibitor (Optional)[1] | 25 units |
| ValueScript Reverse Transcriptase (200 units / µL) | 1 µL |
| RNA template and Primer Mix from Step 2 | 11 µL |
| Nuclease-Free Water | x µL |
| **Total Volume** | **20 µL** |

[1] The use of RNase Inhibitor is optional. It is highly recommended for use with RNA templates of 50 ng or less.

4. 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 |
| 42°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 42°C incubation temperature could be increased to 50-55°C for difficult templates (such as templates with a high degree of secondary structure).

5. 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.