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MonsterScript™ Reverse Transcriptase

Cat. Nos. MSTA5110, and MSTA5124

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1. Introduction

MonsterScript™ Reverse Transcriptase is a thermostable (retains full activity up to 65°C) reverse transcriptase that completely lacks RNase H activity (RNase H$^-$). As a result, the enzyme is highly efficient at producing full-length cDNA from RNA templates ≥15 kb. MonsterScript Reverse Transcriptase is capable of producing cDNA from as little as 1 pg of total RNA for real-time RT-PCR analysis.

MonsterScript Reverse Transcriptase is provided at a concentration of 50 U/µl and is supplied with a 5X Reaction Buffer that provides optimal enzymatic activity. MonsterScript Reverse Transcriptase does not require dithiothreitol (DTT).

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: MonsterScript Reverse Transcriptase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM DTT and 0.1% Triton® X-100.

Unit Definition: One unit converts 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C.

Quality Control: MonsterScript Reverse Transcriptase is function-tested in a cDNA synthesis reaction.

Contaminating Activity Assays: MonsterScript Reverse Transcriptase is free of detectable contaminating DNA exo- and endonuclease and RNase activities.

3. Kit Contents

Cat. #	Concentration	Quantity
MSTA5110		10 Reactions
500 Units	(50 U/µl)	10 µl
MonsterScript™ 5X Reaction Buffer		50 µl
MSTA5124		24 Reactions
1,200 Units	(50 U/µl)	24 µl
MonsterScript™ 5X Reaction Buffer		120 µl

4. Related Products

The following products are also available:

- MonsterScript™ 1st-Strand cDNA Synthesis Kit
- dNTP Solutions
- MasterPure™ RNA Purification Kit
- ArrayPure™ Nano-scale RNA Purification Kit

5. General Considerations for First-Strand cDNA Synthesis

The success of the cDNA synthesis reaction is strongly influenced by the quality of the RNA. Therefore, it is important to prepare and confirm the purity and integrity of the RNA sample prior to beginning the first-strand cDNA synthesis reaction.

1. **RNA Purity:** The RNA sample must be free of salts, metals, ethanol and phenol that are commonly used during purification of the RNA.
2. **RNA Integrity:** The integrity of the RNA can be checked by denaturing agarose gel electrophoresis or by use of the Agilent 2100 Bioanalyzer. RNA that appears degraded by either of these methods should be discarded and a new sample prepared.
3. **Maintaining an RNase-free Environment:** Ribonuclease contamination is a significant concern for those working with RNA. The ubiquitous RNase A is a highly stable and active ribonuclease that can contaminate any lab environment and is present on human skin. The MonsterScript Reverse Transcriptase and MonsterScript 5X Reaction Buffer are both tested to ensure the lack of contaminating ribonuclease activities. However, creating an RNase-free work environment and maintaining RNase-free solutions is critical for performing successful cDNA synthesis reactions. Therefore, we strongly recommend that the user:
    1) Autoclave all tubes and pipette tips that will be used in the cDNA synthesis reactions.
    2) Always wear gloves when handling samples containing RNA. Change gloves frequently especially after touching potential sources of RNase contamination such as door knobs, pens, pencils and human skin.
    3) Always wear gloves when handling kit components. Do not pick up any kit component with an ungloved hand.
    4) Keep all kit components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps.
4. **Choice of Primer for First-Strand cDNA Synthesis:** First-Strand cDNA synthesis can be primed using either Oligo(dT), random or gene-specific primers (all primers provided by the user).

An **Oligo(dT) Primer** is the most commonly used method for priming first-strand cDNA synthesis when using an eukaryotic RNA sample. Oligo(dT) primes cDNA synthesis only from the Poly(A) tail present at the 3′-end of almost all eukaryotic mRNAs. Since Poly(A) RNA constitutes just 1-5% of the RNA in a eukaryotic total cellular RNA preparation, the complexity of the cDNA produced is significantly less than when the cDNA is synthesized using random primers. Lower complexity cDNA can result in a more sensitive and specific PCR reaction. Additionally, priming cDNA synthesis with an Oligo(dT) Primer precludes the need to enrich the RNA sample for Poly(A) RNA.

We recommend a 5′-V_3-Oligo$(dT)_{18\text{-}21}$ primer (V = dA, dC or dG) for most applications using MonsterScript Reverse Transcriptase. The three 5′-'V' nucleotides on this primer significantly reduce primer artifacts that can occur using other primers or enzymes.

Random primers initiate cDNA synthesis from all RNA species (rRNA and mRNA) in a total cellular RNA sample. Since rRNA, which constitutes >95% of the RNA in a total RNA sample, is converted to cDNA using random primers, the complexity of the cDNA will be much greater than when priming the reaction with an Oligo(dT). Random primers, however, can be helpful when:

1) synthesizing cDNA from mRNAs that lack a Poly(A) tail or have a very short Poly(A) tail.
2) priming cDNA synthesis of a Poly(A)-enriched RNA sample.
3) it is necessary to eliminate or reduce 3′-sequence bias that can result when using an Oligo(dT) primer. It should be noted however, that the MonsterScript Reverse Transcriptase is a highly processive enzyme that functions at elevated temperature which greatly enhances full-length cDNA synthesis of even very large (>15 kb) Poly(A) RNA to eliminate 3′-sequence bias even when using the Oligo(dT) primer.

   Gene-specific primers, designed and synthesized by the user, provide the greatest specificity when priming cDNA synthesis of an mRNA. However, the user frequently must determine empirically the optimal primer annealing and extension (reverse transcription) conditions for each primer used.

Procedure

The following protocol has been optimized to convert 1 pg to 1 µg of total cellular RNA to first-strand cDNA using a V_3-Oligo$(dT)_{18\text{-}21}$ or Random Primer(s). Use of gene-specific primers may require additional optimization of the reaction. We recommend a 5′-V_3-Oligo$(dT)_{18\text{-}21}$ primer (V = dA, dC or dG) using MonsterScript Reverse Transcriptase. The three 5′-'V' nucleotides on this primer significantly reduce primer artifacts that can occur using other primers.

MonsterScript 1st-Strand cDNA Synthesis

Gently mix and briefly centrifuge all kit components prior to dispensing.

1. Anneal the selected primer(s) to the RNA sample. For each 1st-strand cDNA synthesis reaction, combine the following components on ice, in a sterile (RNase-free) 0.2 ml or 0.5 ml tube:

x µl	RNase-Free Water
x µl	Total RNA sample (up to 1 µg)
x µl	V_3-Oligo$(dT)_{18\text{-}21}$ Primer (10 pmol)
	- or -
x µl	Random Primers (100 ng)
	- or -
x µl	Gene-specific Primers
10 µl	Total reaction volume

2. Incubate at 65°C for 1 minute in a water bath or thermocycler with heated lid.
3. Chill on ice for 1 minute. Centrifuge briefly in a microcentrifuge.

4. To each 1st-strand cDNA synthesis reaction, add on ice:

1.8	µl	RNase-Free Water
4	µl	MonsterScript 5X Reaction Buffer
0.8	µl	5mM dATP
0.8	µl	5mM dCTP
0.8	µl	5mM dGTP
0.8	µl	5mM dTTP
1	µl	MonsterScript Reverse Transcriptase
20	µl	Total reaction volume

5. Mix the reaction gently.

   If using a V_3-Oligo$(dT)_{18\text{-}21}$ or Oligo$(dT)_{18\text{-}21}$ Primer, proceed directly to Step 6.

   If using Random Primers, incubate the reaction at 37°C for 5 minutes, then proceed to Step 6.
6. Incubate the reaction at 42°C for 5 minutes and then at 60°C for 40 minutes.
7. Terminate the reaction by heating at 90°C for 5 minutes.
8. Chill on ice for at least 1 minute. Centrifuge briefly in a microcentrifuge.
9. The cDNA can be used immediately, without purification, for end-point or real-time PCR or stored at –20°C for future use.

PCR Amplification of the cDNA

Typically, 2 µl (10%) of the 20 µl MonsterScript first-strand cDNA synthesis reaction is sufficient for most 50 µl PCR reactions. However, if detecting a rare mRNA or reverse transcribing a minute amount of total RNA (<100 pg) it is possible to add up to 10 µl (50%) of the 20 µl cDNA synthesis reaction to a 50 µl PCR reaction in order to increase detection sensitivity. Up to 50% of the MonsterScript cDNA synthesis reaction can be added to a PCR reaction because the MonsterScript reaction buffer is more similar to PCR reaction buffers than the reaction buffers used by other reverse transcriptase enzymes.

End-Point PCR: The cDNA produced using MonsterScript Reverse Transcriptase can be used for end-point (standard) PCR.

End-point PCR products are detected by agarose gel electrophoresis and staining with, for example, ethidium bromide or SYBR® Gold. We recommend using Epicentre's FailSafe™ PCR System for end-point PCR of the cDNA. The FailSafe System provides the FailSafe PCR enzyme blend and 12 different FailSafe PCR PreMix Solutions containing dNTPs, buffer and varying amounts of Mg^{+2} and FailSafe PCR Enhancer to enable:

- High specificity PCR without optimization.
- Multiplex PCR.
- Amplification of difficult (e.g. GC-rich) sequences.

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