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MonsterScript™ 1st-Strand cDNA Synthesis Kit

Cat. No. MS040910 – 10 Reactions

Cat. No. MS041050 – 50 Reactions

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1. Introduction

The MonsterScript™ 1st-Strand cDNA Synthesis Kit is optimized for generating full-length first-strand cDNA from total cellular RNA or Poly(A) RNA-enriched samples. The kit features EPICENTRE's MonsterScript Reverse Transcriptase which is capable of synthesizing full-length cDNA from mRNA templates greater than 15 kb.[1]

The ability of MonsterScript Reverse Transcriptase and the MonsterScript 1st-Strand cDNA Synthesis Kit to synthesize long, full-length cDNA derives from four factors:

1. MonsterScript Reverse Transcriptase is a highly processive thermostable enzyme. The thermo-stability of the enzyme permits high temperature reverse transcription in order to reduce the secondary structure of the RNA..
2. MonsterScript Reverse Transcriptase completely lacks RNase H activity (RNase H–).
3. The MonsterScript 5X cDNA PreMix contains betaine* which improves reverse transcription through difficult sequences such as regions of high GC content and secondary structure.
4. The MonsterScript 1st-Strand cDNA Synthesis Kit includes the V_3-Oligo$(dT)_{21}$ Primer. The V_3-Oligo$(dT)_{21}$ Primer significantly reduces primer artifacts that can occur when using a standard Oligo(dT) primer or even an "anchored" Oligo(dT) primer.

2. Kit Contents

	10 rxn	50 rxn
Catalog No.	MS040910	MS041050
MonsterScript™ Reverse Transcriptase (50 U/µl) (with RNase Inhibitor)	10 µl	50 µl
MonsterScript™ 5X cDNA PreMix (contains buffer, dNTPs and betaine*)	50 µl	250 µl
V_3-Oligo$(dT)_{21}$ Primer (10 µM)	10 µl	50 µl
Random 9-mer Primers (50 µM)	20 µl	100 µl
RNase-Free Water	1 ml	1 ml

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

Additionally Required Reagents and Equipment: Water bath or thermocycler

3. Related Products

The following products are also available:

– MonsterScript™ Reverse Transcriptase
– dNTP Solutions
– MasterPure™ RNA Purification Kit
– ArrayPure™ Nano-scale RNA Purification Kit

4. Performance Specifications and Quality Control

The MonsterScript 1st-Strand cDNA Synthesis Kit is function-tested in a control reaction using the V3-Oligo(dT) primer. In this reaction, the kit converts an ~2 kb poly(A) RNA into full length cDNA in 30 minutes at 60°C.

Assessing the Quality of the Total RNA

The success of the cDNA synthesis reaction, and subsequent PCR reaction, is strongly influenced by the quality of the RNA. RNA quality has two components…purity of the RNA (or absence of contaminants) and integrity (intactness) of the RNA. RNA quality should be assessed prior to every cDNA synthesis reaction.

RNA Purification Methods and RNA Purity

Total cellular RNA, isolated by a number of methods, can be reverse transcribed successfully using the MonsterScript 1st-Strand cDNA Synthesis Kit. However, it is very important that the purified RNA be free of salts, metal ions, ethanol and phenol which can inhibit the enzymatic reactions performed in the reverse transcription process. Commonly used RNA extraction and purification methods that are compatible with the MonsterScript reverse transcription process include but are not limited to:

TRIzol® / TRI Reagent®, a homogeneous solution of the powerful denaturants guanidinium iso-thiocyanate and phenol, is very effective at extracting the RNA from the cells. However all traces of guanidinium salts and phenol must be removed from the RNA sample prior to the RNA amplification process. If you precipitate the RNA from TRIzol-extracted cells, be sure to wash the RNA pellet at least two times with cold 70-75% ethanol to remove all traces of phenol and guanidinium salts. Air dry the RNA pellet (do not use a vacuum centrifuge) to remove residual ethanol. Then, resuspend the RNA in RNase-Free water. If you purify the RNA from TRIzol-extracted cells by column purification methods, please read the section "Spin Columns" immediately following.

Spin Columns (e.g., the RNeasy® MinElute Cleanup Kit and RNeasy Mini Kit from Qiagen) are effective in purifying RNA samples that are free of the contaminants that may inhibit the reverse transcription reaction. Spin columns can be used with most RNA extraction procedures (e.g. TRIzol reagent). If using spin columns, follow the manufacturer's instructions closely, especially if an ethanol wash of the RNA is performed prior to the RNA elution step. Then, elute the RNA from the column membrane using RNase-Free water.

Salt-Fractionation: RNA purification that employs gentle salt-fractionation, such as EPICENTRE's MasterPure™ RNA Purification Kit and ArrayPure™ Nano-Scale RNA Purification Kit, routinely produce the highest yield of intact RNA without the use of phenol, guanidinium salts or columns. The ArrayPure kit has been developed for total RNA purification from 1-10,000 cells obtained by laser-capture methods, from biopsy samples, from cell culture or quick-frozen tissue. To purify RNA from >10,000 cells, Epicentre's MasterPure RNA Purification Kit is recommended. When using these kits, be sure to wash the RNA pellet at least two times with cold 70-75% ethanol to remove all traces of salts. Air dry the RNA pellet (do not use a vacuum centrifuge) to remove residual ethanol. Then, resuspend the RNA in RNase-Free water. The ArrayPure kit and MasterPure kit are not recommended for purification of RNA from tissue samples preserved with RNAlater® or RNAlater-ICE.

RNA Integrity

Synthesis of full-length cDNA is dependent on an RNA sample that contains intact Poly(A) RNA. Presently, the most frequently used methods for assaying RNA integrity are by dena-turing agarose gel electrophoresis or using an Agilent 2100 Bioanalyzer.

The advantages of denaturing agarose gel electrophoresis are its low cost and ready availability of the reagents required. Denaturing gel electrophoresis separates the RNAs by size (electrophoretic mobility) under denaturing conditions. Denaturing conditions are necessary to eliminate inter- and intra-molecular secondary structure within the RNA sample which may cause degraded RNA to appear intact. Following electrophoresis, the denaturing gel is stained with, for example, ethidium bromide, when using a eukaryotic RNA sample, the user looks for the highly stained 18S and 28S rRNAs. These bands should be sharp and discrete with an absence of smearing under either. Based on these visual observations, the user infers that the mRNA in the sample is equally intact. If the rRNA bands appear degraded, as evidenced by smearing under each, the RNA sample should be discarded and a new sample of total RNA purified. Ideally, the ethidium bromide stained 28S rRNA band should appear to be about twice as intense as the 18S rRNA band.

The Agilent 2100 Bioanalyzer is currently the preferred method for evaluating the integrity of an RNA sample. Like a denaturing gel, the bioanalyzer separates the RNAs by size (electrophoretic mobility). However, in contrast to a denaturing gel, the 2100 Bioanalyzer consumes as little as 5 ng of total RNA per well when using the manufacturer's RNA 6000 Nano LabChip®. When analyzing the RNA sample using the Agilent 2100 Bioanalyzer, the 18S and 28S rRNA species should appear as distinct, sharp peaks on the electropherogram. A slightly increased baseline, indicative of the 1-5% Poly(A) RNA contained in the sample, can be seen between the two peaks.

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. All components of the MonsterScript 1st-Strand cDNA Synthesis Kit have been 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 espe-cially after touching potential sources of RNase contamination such as door knobs, pens, pen-cils 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.

Choice of Primer for First-Strand cDNA Synthesis

First-Strand cDNA synthesis can be primed using either the V_3-Oligo(dT)$_{21}$ Primer or Random Nona-mer (9-mer) Primers that are provided in the kit or using gene-specific primers (provided by the user).

An Oligo(dT) primer is the most commonly used method for priming first-strand cDNA synthesis from an eukaryotic RNA sample. Oligo(dT) primes cDNA synthesis from the Poly(A) tail present at the 3′-end of almost all eukaryotic mRNAs. Priming cDNA synthesis with an Oligo(dT) Primer precludes the need to enrich the RNA sample for Poly(A) RNA. The V_3-Oligo(dT)$_{21}$ Primer, included in the MonsterScript 1st-Strand cDNA Synthesis Kit, differs from both standard and "anchored" Oligo(dT) primers. The V_3-Oligo(dT)$_{21}$ Primer is a proprietary primer discovered by Epicentre scientists that significantly reduces primer artifacts when using MonsterScript Reverse Transcriptase. We recommend using the V_3-Oligo(dT)$_{21}$ Primer for most applications.

Random Nonamer (9-mer) Primers initiate cDNA synthesis from all RNA species (rRNA and mRNA) contained 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 resulting cDNA will be much greater than when priming the reaction with an Oligo(dT) primer. The more complex cDNA sample can result in reduced sensitivity and specificity of the subsequent PCR reaction. Random primers, however, can be helpful when:

1) synthesizing cDNA from eukaryotic 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 included in the kit is a highly processive enzyme that functions at elevated temperature and that the MonsterScript PreMix contains betaine*…all of which greatly enhance full-length cDNA synthesis of even very large (> 15 kb) Poly(A) RNA and help to eliminate 3′-sequence bias.

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.

RT-PCR: End-Point or Real-Time PCR Amplification and Detection

The cDNA product of a MonsterScript 1st-Strand cDNA Synthesis Kit reaction can be used in either end-point (standard) or real-time PCR. Detection of real-time PCR products is significantly more sensitive than detection of PCR products from end-point PCR reaction. Therefore, the amount of total RNA to use in a MonsterScript 1st-Strand cDNA Synthesis Kit reaction may be dependent on the type of PCR the user intends to perform.

End-point PCR products are detected by agarose gel electrophoresis and staining with, for example, ethidium bromide or SYBR® Gold. We recommend using at least 10 ng of total RNA in the MonsterScript 1st-Strand cDNA Synthesis reaction when end-point PCR will be performed.

Real-time PCR is much more sensitive than end-point PCR. When performing real-time PCR, as little as 1 pg of total RNA can be used in the MonsterScript 1st-Strand cDNA Synthesis Kit reaction.

5. Procedure

The following protocol has been optimized to convert 1 pg to 1 µg of total cellular RNA to first-strand cDNA using the V_3-Oligo$(dT)_{21}$ Primer or Random Nonamer (9-mer) Primers provided in the kit. Gene-specific primers (provided by the user) may require additional optimization of the reaction.

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)
1	µl	V_3-Oligo$(dT)_{21}$ Primer (10 µM)
		- or -
2	µl	Random 9-mer Primers (50 µM)
		- or -
x	µl	Gene-specific Primers
15	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:

4	µl	MonsterScript 5X cDNA PreMix
1	µl	MonsterScript Reverse Transcriptase

5. Mix the reaction gently.
   If using the V_3-Oligo$(dT)_{21}$ Primer, proceed directly to Step 6.
   If using the Random 9-mer 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 1st-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 re-action to a 50 µl PCR reaction in order to increase detection sensitivity. Up to 50% of the Monster-Script 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. The cDNA produced using the MonsterScript Reverse Transcriptase can be used for end-point (standard) PCR or real-time 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 (with betaine*) to enable:

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

6. Reference

1. Vaidyanathan, R. (2005) *Epicentre Forum* **12** (1).

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