SuperScript™ First-Strand Synthesis System for RT-PCR

Cat. No: 11904-018 **Size: 50 reactions**

Store at -20°C

Description

The SuperScript™ First-Strand Synthesis System for RT-PCR is optimized to synthesize first-strand cDNA from purified poly(A)+ or total RNA. The system can be used with as little as 1 ng or as much as 5 µg of total RNA. After synthesis, the cDNA can be amplified with specific primers by PCR without intermediate organic extractions or ethanol precipitations.

The first-strand cDNA synthesis reaction is catalyzed by SuperScript™ II Reverse Transcriptase (RT). This enzyme has been engineered to reduce the RNase H activity that degrades mRNA during the first-strand reaction, resulting in greater full-length cDNA synthesis and higher yields of first-strand cDNA than obtained with RNase H^+ RTs. Because SuperScript™ II RT is not inhibited significantly by ribosomal and transfer RNA, it may be used effectively to synthesize first- strand cDNA from a total RNA preparation. The enzyme exhibits increased thermal stability and may be used at temperatures up to 50°C.

This system has been optimized to synthesize first-strand cDNA from varying amounts of starting material. The SuperScript™ II RT concentration has been lowered and RNaseOUT™ Recombinant RNase Inhibitor has been added to the system as part of this optimization process. Additionally, reaction conditions have been modified to further increase the sensitivity of the system.

Using the kit, you synthesize first-strand cDNA using either total RNA or poly(A)$^+$-selected RNA primed with oligo(dT), random primers, or a gene-specific primer. Then you perform PCR in a separate tube using primers specific for the gene of interest.

Kit Components

Component	Amount
Oligo(dT)$_{12–18}$ (0.5 µg/µl)	50 µl
Random hexamers (50 ng/µl)	250 µl
10X RT buffer*	1 ml
25 mM $MgCl_2$	500 µl
0.1 M DTT	250 µl
10 mM dNTP mix	250 µl
SuperScript™ II RT (50 U/µl)	50 µl
RNaseOUT™ (40 U/µl)	100 µl
E. coli RNase H (2 U/µl)	50 µl
DEPC-treated water	1.2 ml
Control RNA (50 ng/µl)	15 µl
Control Primer A (10 µM)	20 µl
Control Primer B (10 µM)	20 µl

*200 mM Tris-HCl (pH 8.4), 500 mM KCl

Additional Products

The following related products are available from Invitrogen. Visit our online catalog at www.invitrogen.com to order.

Product	Amount	Catalog No.
PureLink™ Micro-to-Midi™ Total RNA Purification System	50 rxns	12183-018
TRIzol® Reagent	100 ml	15596-026
	200 ml	15596-018
PureLink™ 96 RNA Purification System	4 × 96 rxns	12173-011
DNase I, Amplification Grade	100 units	18068-015
Taq DNA Polymerase, native (5 U/µl)	100 units	18038-018
	500 units	18038-042
Taq DNA Polymerase, recombinant (5 U/µl)	100 units	10342-053
	500 units	10342-020
Platinum® *Taq* DNA Polymerase	100 rxns	10966-018
	250 rxns	10966-026
	500 rxns	10966-034
Platinum® *Taq* DNA Polymerase High Fidelity	100 rxns	11304-011
	500 rxns	11304-029
Platinum® *Pfx* DNA Polymerase	100 rxns	11708-013
	250 rxns	11708-021
Pfx50™ DNA Polymerase	100 rxns	12355-012
PCR_x Enhancer System	250 rxns	11495-017
Custom Primers	to order, visit www.invitrogen.com	

Additional Materials Required

The following materials are supplied by the user:

- Isolated total RNA or mRNA
- Optional: DNase I, Amplification Grade
- DNA polymerase for PCR
- Programmable thermal cycler
- Sterile 0.2- or 0.5-ml microcentrifuge tubes or sterile, thin-walled PCR tubes
- Automatic pipettes and tips capable of dispensing 1–20 µl and 20–200 µl
- Disposable gloves
- Two amplification primers specific for your target mRNA
- Microcentrifuge capable of generating a relative centrifugal force of 14,000 × *g*
- Water baths, heat blocks, or incubators at 37°C, 42°C, 65°C, and 70°C

Part no. 11904.pps Rev. date: 5 Mar 2007

Recommendations and Guidelines for First-Strand Synthesis

RNA

- High-quality, intact RNA is essential for full-length, high-quality cDNA synthesis. This kit is designed for use with 1 ng to 5 µg of total RNA or 50 to 500 ng of poly(A)$^+$ RNA. If you have >5 µg of total RNA, increase reaction volumes and amount of SuperScript™ II RT proportionally.
- RNaseOUT™ Recombinant RNase Inhibitor is included with the system to safeguard against degradation of target RNA due to ribonuclease contamination.
- To isolate total RNA, we recommend the PureLink™ Micro-to-Midi™ Total RNA Purification System, TRIzol® Reagent, or the PureLink™ 96 Total RNA Purification Kit for high-throughput applications. Isolation of mRNA is typically not necessary, although incorporating this step may improve the yield of specific cDNAs and reduce the likelihood of genomic DNA contamination.
- Small amounts of genomic DNA in the RNA preparation may be amplified along with the target cDNA. If your application requires removal of all genomic DNA from your RNA preparation, we recommend using DNase I, Amplification Grade (Catalog no. 18068-015).

RNase H Digestion

The sensitivity of the PCR step can be increased (especially for long templates) by removing the RNA template from the cDNA:RNA hybrid molecule by digestion with RNase H after first-strand synthesis. Presence of RNase H during first-strand synthesis will degrade the template mRNA, resulting in decreased full-length cDNA synthesis and decreased yields of first-strand cDNA. The SuperScript™ First-Strand Synthesis System introduces RNase H activity only when it is beneficial.

Primers

The first-strand cDNA synthesis reaction can be primed using random hexamers, oligo(dT), or gene-specific primers (GSPs):

- Random hexamers are the most nonspecific priming method, and are typically used when the mRNA is difficult to copy in its entirety. With this method, all RNAs in a population are templates for first-strand cDNA synthesis, and PCR primers confer specificity during PCR. To maximize the size of cDNA, you should determine the ratio of random hexamers to RNA empirically for each RNA preparation.

  Note: For most RT-PCR applications, 50 ng of random hexamers per 5 µg of total RNA is adequate. Increasing hexamers to 250 ng per 5 µg of RNA may increase yield of small PCR products (<500 bp), but may decrease the yield of longer PCR products and full-length transcripts.
- Oligo(dT), a more specific priming method, is used to hybridize to 3´ poly(A) tails, which are found in the vast majority of eukaryotic mRNAs. Since poly(A)$^+$ RNA constitutes approximately 1% to 2% of total RNA, the amount and complexity of cDNA is considerably less than with random hexamers.

  Note: Oligo(dT) is recommended over random hexamers or GSPs when performing RT-PCR with new mRNA targets. Oligo(dT) produces an RT-PCR product more consistently than random hexamers or GSPs.
- The most specific priming method uses a gene-specific primer (GSP) for the sequence of interest. First-strand synthesis can be primed with the PCR primer that hybridizes nearest to the 3´ terminus of the mRNA. Note that some GSPs fail to prime cDNA synthesis even though they work in PCR on DNA templates. If gene-specific priming fails in RT-PCR, repeat first-strand synthesis using oligo(dT) as the primer.

First-Strand Synthesis Using Oligo(dT) or GSP

Starting material: 1 ng–5 µg total RNA or 50–500 ng poly(A)$^+$ RNA

Control reactions: Use 1 µl of Control RNA (50 ng/µl)

1. Mix and briefly centrifuge each component before use.
2. For each reaction, combine the following in a sterile 0.2- or 0.5-ml tube:

Component	Amount
RNA	*n* µl
10 mM dNTP mix	1 µl
Primer (0.5 µg/µl oligo(dT)$_{12–18}$, *or* 2 µM gene-specific primer)	1 µl
DEPC-treated water	to 10 µl

3. Incubate the RNA/primer mixture at 65°C for 5 minutes, then place on ice for at least 1 minute.
4. In a separate tube, prepare the following 2X reaction mix, adding each component in the indicated order.

Component	1 Rxn	10 Rxns
10X RT buffer	2 µl	20 µl
25 mM $MgCl_2$	4 µl	40 µl
0.1 M DTT	2 µl	20 µl
RNaseOUT™ (40 U/µl)	1 µl	10 µl

5. Add 9 µl of the 2X reaction mix to each RNA/primer mixture from step 3, mix gently, and collect by brief centrifugation.
6. Incubate at 42°C for 2 minutes.
7. Add 1 µl of SuperScript™ II RT to each tube. **Minus RT Control:** Add 1 µl DEPC-treated water instead of the RT.
8. Incubate at 42°C for 50 minutes.
9. Terminate the reaction at 70°C for 15 minutes. Chill on ice.
10. Collect the reaction by brief centrifugation. Add 1 µl of RNase H to each tube and incubate for 20 minutes at 37°C.

The reaction can be stored at -20°C or used for PCR immediately.

First-Strand Synthesis Using Random Primers

Starting material: 1 ng–5 µg total RNA or 50–500 ng poly(A)$^+$ RNA

Control reactions: Use 1 µl of Control RNA (50 ng/µl)

1. Mix and briefly centrifuge each component before use.
2. For each reaction, combine the following in a sterile 0.5-ml tube:

Component	Amount
RNA	*n* µl
10 mM dNTP mix	1 µl
Random hexamers (50 ng/µl)	1–5 µl
DEPC-treated water	to 10 µl

3. Incubate the RNA/primer mixture at 65°C for 5 minutes, then place on ice for at least 1 minute.
4. In a separate tube, prepare the following 2X reaction mix, adding each component in the indicated order.

Component	1 Rxn	10 Rxns
10X RT buffer	2 µl	20 µl
25 mM $MgCl_2$	4 µl	40 µl
0.1 M DTT	2 µl	20 µl
RNaseOUT™ (40 U/µl)	1 µl	10 µl

5. Add 9 µl of the 2X reaction mix to each RNA/primer mixture from step 3, mix gently, and collect by brief centrifugation.
6. Incubate at room temperature (~25°C) for 2 minutes.
7. Add 1 µl of SuperScript™ II RT to each tube. **Minus RT Control:** Add 1 µl DEPC-treated water instead of the RT.
8. Incubate at room temperature for 10 minutes.
9. Incubate at 42°C for 50 minutes.
10. Terminate the reaction at 70°C for 15 minutes. Chill on ice.
11. Collect the reaction by brief centrifugation. Add 1 µl of RNase H to each tube and incubate for 20 minutes at 37°C.

The reaction can be stored at -20°C or used for PCR immediately.

Control Reactions

Control Primer A (antisense) and Control Primer B (sense) provided with this kit are designed to produce a 500-bp RT-PCR product from the Control RNA.

Control Primer A: 5'-GAC ATG GAA GCC ATC ACA GAC-3'
Control Primer B: 5'-AGA CCG TTC AGC TGG ATA TTA C-3'

Use the following protocol for both plus and minus RT control reactions:

1. Prepare serial dilutions of the cDNA from the control first-strand reaction on page 2. Dilute at 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000.

   Note: If you assume that 1% of total RNA is mRNA, then 50 ng of Control RNA in the first-strand reaction is equivalent to the amount of mRNA in 5 µg of total RNA. One microliter of the 1:1,000,000 dilution of the first-strand reaction should contain ~5,000 molecules of cDNA.
2. For each dilution, prepare a PCR mixture. Add the following to a 0.2-ml tube sitting on ice:

Component	Volume
10X PCR buffer minus Mg^{++}	5 µl
25 mM $MgCl_2$	3 µl
10 mM dNTP mix	1 µl
Control Primer A (10 µM)	1 µl
Control Primer B (10 µM)	1 µl
Diluted cDNA from control reaction	1 µl
Taq DNA polymerase (5 units/µl)	0.4 µl
DEPC-treated water	to 50 µl

3. Mix the contents of the tube. Centrifuge briefly to collect the reaction components.
4. Place reaction mixture in preheated (94°C) thermal cycler. Perform an initial denaturation step: 94°C for 2 minute.
5. Perform 35 cycles of PCR:

   Denature 94°C for 15 seconds
   Anneal 55°C for 30 seconds
   Extend 68°C for 1 minute

   Note: For slow-ramping thermal cyclers, follow manufacturer's directions.
6. Upon completion, maintain the reaction at 4°C.
7. Analyze 10 µl of each reaction, using agarose gel electrophoresis. For each Plus RT Control reaction, a 500-bp band, corresponding to at least 25 ng of product, should be visible. For the Minus RT Control reaction, the same band should be ≤50% in intensity when compared to the Plus RT Control.

First Strand Synthesis of Transcripts with High GC Content

High-GC content mRNAs can contain stable secondary structures that can inhibit reverse transcriptase and/or primer annealing. These problems often can be overcome by increasing the volume and temperature of the RT reaction.

Note: For templates that require cDNA synthesis temperatures of 42–55°C, we recommend SuperScript™ III RT. For cDNA synthesis above 55°C, we recommend Thermo-X™ Reverse Transcriptase (Catalog no. 11150-100). Thermo-X™ RT supports cDNA synthesis up to 70°C.

To avoid secondary RNA structure using SuperScript™ II RT, transfer the RNA/primer mix directly from 65°C to 50°C and prewarm the complete 2X reaction mix to 50°C before adding it to the primer and RNA. Using a thermal cycler simplifies the multiple temperature shifts and can help prevent formation of secondary structure in RNA.

This protocol is suitable for gene-specific or oligo(dT) primers, but not random hexamers.

1. Mix and briefly centrifuge each component before use.
2. Prepare the RNA/primer mixture in a sterile 0.5-ml tube as follows:

Component	Sample	Control RNA
RNA	*n* µl	—
Control RNA (50 ng/µl)	—	1 µl
Oligo(dT) $_{12–18}$ (0.5 ng/µl) *or* 2 µM GSP	1 µl	1 µl
10 mM dNTP mix	2.5 µl	2.5 µl
DEPC-treated water	to 25 µl	to 25 µl

3. Incubate the RNA/primer mixture at 65°C for 5 minutes and immediately transfer to 50°C.
4. In a separate tube, prepare the 2X reaction mix, adding each component in the indicated order.

Component	1 Reaction	10 Reactions
DEPC-treated water	4 µl	40 µl
10X RT buffer	5 µl	50 µl
25 mM $MgCl_2$	10 µl	100 µl
0.1 M DTT	5 µl	50 µl
RNaseOUT™	1 µl	10 µl

5. Prewarm the 2X reaction mix to 50°C.
6. To each RNA/primer mixture from Step 3 incubating at 50°C, add 25 µl of prewarmed 2X reaction mix.
7. Add 1 µl of SuperScript™ II RT to each tube. **Minus RT Control:** Add 1 µl DEPC-treated water instead of the RT.
8. Mix gently, and incubate at 50°C for 50 minutes.
9. Terminate the reaction at 70°C for 15 minutes. Chill on ice.
10. Collect the reaction by brief centrifugation. Add 1 µl of RNase H to each tube and incubate for 20 minutes at 37°C before proceeding to PCR.

Note: Frequently, problems associated with RT-PCR of GC-rich cDNA are related to PCR as well as first-strand synthesis. PCR_x Enhancer System, a PCR cosolvent (Catalog no. 11495-017), can facilitate amplification of GC-rich sequences.

Amplification of Target cDNA

The cDNA synthesized in the first-strand reaction may be amplified directly using PCR. We recommend using 10% of the first-strand reaction (2 µl) for PCR. For some targets, increasing the amount of cDNA in PCR up to 10 µl may result in increased product yield. In addition to *Taq* DNA Polymerase, Invitrogen offers the following DNA polymerases for high-specificity and/or high-fidelity PCR:

- **Platinum® *Taq* DNA Polymerase** provides automatic hot-start conditions for increased specificity up to 4 kb
- **Platinum® *Taq* DNA Polymerase High Fidelity** provides increased yield and high fidelity for targets up to 15 kb
- **Platinum® *Pfx* DNA Polymerase** provides very high fidelity (25X compared to *Taq* DNA Polymerase) for targets up to 12 kb
- ***Pfx50*™ DNA Polymerase** provides maximum fidelity (50X compared to *Taq* DNA Polymerase) for targets up to 4 kb

Consult the product documentation provided with each DNA polymerase for recommended protocols and optimization guidelines. Documentation is also available at www.invitrogen.com.

Troubleshooting Guide

Problem	Possible Cause	Probable Solution
No bands after analysis of amplified products	Procedural error in first-strand cDNA synthesis	Use the Control RNA to verify the efficiency of the first-strand reaction.
	RNase contamination	Add control RNA to sample to determine if RNase is present in the first-strand reaction. Maintain aseptic conditions to prevent RNase contamination. Use RNaseOUT™ Recombinant RNase Inhibitor in the first-strand reaction.
	Polysaccharide coprecipitation of RNA	Precipitate RNA with lithium chloride to remove polysaccharides, as described in Sambrook *et al.*
	Target mRNA contains strong transcriptional pauses	Use random hexamers instead of oligo(dT) in the first-strand reaction. Maintain an elevated temperature after the annealing step, as described in the protocol for first-strand synthesis from high-GC content transcripts, page 3. Increase the temperature of first-strand reaction (up to 50°C). Use PCR primers closer to the 3´ terminus of the target cDNA.
	Too little first-strand product was used in PCR	Use up to 10 µl of the first-strand reaction.
	GSP was used for first-strand synthesis	Try another GSP or switch to oligo(dT). Make sure the GSP is the antisense sequence.
	Inhibitors of RT present	Remove inhibitors by ethanol precipitation of mRNA preparation before the first-strand reaction. Include a 70% (v/v) ethanol wash of the mRNA pellet. **Note:** Inhibitors of RT include sodium dodecyl sulfate (SDS), EDTA, guanidinium salts, formamide, sodium pyrophosphate, and spermidine.
Unexpected bands after electrophoretic analysis	Contamination by genomic DNA	Pretreat RNA with DNase I, Amplification Grade (Cat. no. 18068-015), as described in the DNase I documentation. Design primers that anneal to sequence in exons on both sides of an intron or at the exon/exon boundary of the mRNA to differentiate between amplified cDNA and potential contaminating genomic DNA. To test if products were derived from DNA, perform the minus RT control.
	Nonspecific annealing of primers	Vary the annealing conditions. Use Platinum® *Taq* DNA Polymerase for automatic hot-start PCR. Optimize magnesium concentration for each template and primer combination.
	Primers formed dimers	Design primers without complementary sequences at the 3´ ends.

References

Berger, S.L. and Kimmel, A.R. (1987) *Methods Enzymol* 152, 316.
Bracete, A.M., Mertz, L.M., Fox, D.K. (1999) *Focus*® 21, 38.
Chomczynski, P. (1993) *Biotechniques* Vol. 15, 532.
Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* 162, 156.
Compton, T. (1990) in *PCR Protocols: A Guide to Methods and Applications* (Innis, M., Gelfand, D., Sninsky, J., and White, T., eds.), p. 39, Academic Press, Inc.
Frohman, M.A., Dush, M.K, and Martin, G.R. (1988) *Proc. Nat. Acad. Sci USA* 85, 8998.
Gerard, G.F. (1994) *Focus*® 16, 102.
Sambrook J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
Simms, D., Cizdziel, P.E., and Chomczynski, P. (1993) *Focus*® 15, 99.
Westfall, B., Sitaraman, K., Solus, J., Hughes, J., and Rashtchian, A. (1997) *Focus*® 19, 46.
Westfall, B., Sitaraman, K., Lee, J., Borman, J. and Rashtchian, A. (1999) *Focus*® 21, 49.
Takagi, M., Nishioka, M., Kakihara, H., Kitabayashi, M., Inoue, H., Kawakami, B., Oka, M., and Imanaka, T. (1997) *Appl. Environ. Microbiol.* 63, 4504.
Sitaraman, K., Darfler, M., and Westfall, B. (1999) *Focus*® 21, 10.
Nathan, M., Mertz, L., Fox, D. (1995) *Focus*® 17, 78.
Schwabe, W., Lee, J.E., Nathan, M., Xu, R.H., Sitaraman, K., Smith, M., Potter, R.J., Rosenthal, K., Rashtchian, A., Gerard, G.F. (1998) *Focus*® 20, 30.

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