# SuperScript® III First-Strand Synthesis SuperMix

**Cat. No: 18080-400** **Size: 50 reactions**

**Store at –20°C**

## Description

The SuperScript® III First-Strand Synthesis System SuperMix is an optimized SuperMix formulation for first-strand cDNA synthesis from purified poly(A)$^+$ or total RNA. RNA targets from 100 bp to >12 kb can be detected with this system. The amount of starting material can vary from 0.1 pg to 5 µg of total RNA.

The kit includes SuperScript® III/RNaseOUT™ Enzyme Mix, 2X First-Strand Reaction Mix, and Annealing Buffer. SuperScript® III Reverse Transcriptase. Included in the Enzyme Mix, is a version of M-MLV RT that has been engineered to reduce RNase H activity and provide increased thermal stability (1, 2). The enzyme can be used to synthesize cDNA at a temperature range of 45–55°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases. Because SuperScript® III RT is not significantly inhibited by ribosomal and transfer RNA, it can be used to synthesize cDNA from total RNA.

RNaseOUT™ Recombinant RNase Inhibitor is included in the Enzyme Mix to safeguard against degradation of target RNA due to ribonuclease contamination.

The 2X First-Strand Reaction Mix includes 10 mM $MgCl_2$ and 1 mM of each dNTP in a buffer formulation that has been optimized for first-strand synthesis of cDNA. The Annealing Buffer is used in the initial template-primer annealing step. Separate tubes of oligo(dT)$_{20}$ and random hexamers are also provided.

cDNA synthesis can be performed using either total RNA or poly(A)$^+$-selected RNA primed with oligo(dT), random primers, or a gene-specific primer. Recommendations for PCR amplification of the first-strand cDNA are provided on page 3.

## Kit Contents

| Component | Amount |
|---|---|
| SuperScript® III/RNaseOUT™ Enzyme Mix | 100 µL |
| 2X First-Strand Reaction Mix (contains 10 mM $MgCl_2$, and 1 mM each dNTP) | 500 µL |
| Annealing Buffer | 50 µL |
| Oligo(dT)$_{20}$ (50 µM) | 50 µL |
| Random hexamers (50 ng/µL) | 50 µL |

## Related Products

| Product | Amount | Catalog No. |
|---|---|---|
| Platinum® *Taq* DNA Polymerase | 100 units | 10966-018 |
| | 250 units | 10966-026 |
| | 500 units | 10966-034 |
| Platinum® *Taq* DNA Polymerase High Fidelity | 100 units | 11304-011 |
| | 500 units | 11304-029 |
| Platinum® *Pfx* DNA Polymerase | 100 units | 11708-013 |
| | 250 units | 11708-021 |
| | 500 units | 11708-039 |
| PureLink™ RNA Mini Kit | 50 rxns | 12183-018A |
| TRIzol® Reagent | 100 mL | 15596-026 |
| | 200 mL | 15596-018 |
| FastTrack® 2.0 mRNA Isolation Kit | 6 reactions | K1593-02 |
| DNase I, Amplification Grade | 100 units | 18068-015 |
| Custom Primers | to order, visit www.invitrogen.com | |

## Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

## Product Qualification

The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website at www.invitrogen.com/support.

## Recommendations and Guidelines

### Required Materials

- 0.2-mL, nuclease-free thin-walled PCR tubes
- Thermal cycler, preheated to 65°C
- Ice
- RNase/DNase-free water
- Microcentrifuge
- Vortex mixer

### RNA

- High-quality, intact RNA is essential for full-length, high-quality cDNA synthesis. This kit is designed for use with 0.1 pg to 5 µg of total RNA or 0.1 pg to 500 ng of poly(A)$^+$ RNA.
- RNaseOUT™ Recombinant RNase Inhibitor is included in the Enzyme Mix to safeguard against degradation of target RNA due to ribonuclease contamination of the RNA preparation.
- To isolate total RNA, we recommend TRIzol® Reagent (Cat. nos. 15596-026/-018) or the PureLink™ RNA Mini-Kit (Cat. no. 12183-018A). Isolation of poly(A)$^+$ RNA is typically not necessary, although it may improve the yield of specific cDNAs; we recommend the FastTrack® 2.0 mRNA Isolation Kit (Cat. no. K1593-02).
- 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 (Cat. no. 18068-015). DNase I, Amplification Grade, has been extensively purified to remove trace ribonuclease activities commonly associated with other "RNase-free" enzyme preparations, and does not require the addition of placental RNase inhibitor.

### cDNA Synthesis Reaction Conditions

- For difficult or high GC-content templates, use a 55°C cDNA synthesis temperature.
- Keep all components, reaction mixes, and samples on ice. After preparation of the annealing mixture, transfer the mixture to the preheated thermal cycler.

### Primers

First-strand cDNA synthesis 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 for difficult or high GC-content mRNA. Using random hexamers, all RNAs in a population are templates for first-strand cDNA synthesis, and PCR primers confer specificity during PCR.

  **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. We recommend using the oligo(dT)$_{20}$ provided in the kit.

  **Note:** Oligo(dT) is recommended over random hexamers or GSPs for 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.

### Guidelines for PCR

The first-strand cDNA from the synthesis reaction may be amplified directly using PCR. We recommend using 10% of the first-strand reaction (2 µL) for PCR. However, for some targets, increasing the amount of first-strand reaction to up to 10 µL may result in increased product yield.

We recommend the following DNA polymerases (for ordering information, see page 1):

- **Platinum® *Taq* DNA Polymerase** provides automatic hot-start conditions for increased specificity and sensitivity. It is recommended for targets up to 4 kb.
- **Platinum® *Taq* DNA Polymerase High Fidelity** provides increased fidelity and higher yields for targets up to 15 kb.
- **Platinum® *Pfx* DNA Polymerase** possesses a proofreading 3´ to 5´ exonuclease activity and provides maximum fidelity for PCR. It is recommended for targets up to 12 kb.

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

## First-Strand cDNA Synthesis

The following procedure is designed to convert 0.1 pg to 5 µg of total RNA or 0.1 pg to 500 ng of poly(A)$^+$ RNA into first-strand cDNA:

1. Mix and briefly centrifuge each component before use. Preheat the thermal cycler to 65°C.
2. Combine the following in a 0.2-mL thin-walled PCR tube on ice:

| Component | Amount |
|---|---|
| up to 5 µg total RNA | *n* µL |
| Primer (50 µM oligo(dT)$_{20}$, *or* 2 µM gene-specific primer, *or* 50 ng/µL random hexamers) | 1 µL |
| Annealing Buffer | 1 µL |
| RNase/DNase-free water | to 8 µL |

3. Incubate in a thermal cycler at 65°C for 5 minutes, and then immediately place on ice for at least 1 minute. Collect the contents of the tube by brief centrifugation.
4. Add the following to the tube on ice:

| | |
|---|---|
| 2X First-Strand Reaction Mix | 10 µL |
| SuperScript® III/RNaseOUT™ Enzyme Mix | 2 µL |

5. Vortex the sample briefly to mix, and collect by brief centrifugation. Incubate as follows:

   Oligo(dT)$_{20}$ or GSP primed: 50 minutes at 50°C

   Random hexamer primed: 5–10 minutes at 25°C, followed by 50 minutes at 50°C
6. Terminate the reactions at 85°C for 5 minutes. Chill on ice.

Store the cDNA synthesis reaction at –20°C, or proceed directly to PCR.

## Troubleshooting Guide

| Problem | Probable Solution |
|---|---|
| **No or faint bands after analysis of amplified products** | |
| Procedural error in first-strand cDNA synthesis | Repeat the procedure, being careful to follow each step. Be careful to include the Annealing Buffer when adding primers and template for optimal yield. |
| RNase contamination | Maintain aseptic conditions to prevent RNase contamination. RNaseOUT™ is included in the Enzyme Mix to inhibit RNases. |
| 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. |
| | Increase the reaction temperature to 55°C. |
| | Prewarm the 2X First-Strand Reaction Mix to 55°C and hold the primer-template mixture at 55°C, then add the Enzyme Mix. Perform cDNA synthesis at 55°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. |
| 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


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