Invitrogen™
life technologies

SuperScript™ III RTS First-Strand cDNA Synthesis Kit

Catalog No.	Format:	Store at room temperature
18580-008	12 × 8 strip wells, plate format	
18580-096	96-well plate	

Description

The SuperScript™ III RTS First-Strand cDNA Synthesis Kit provides qualified reagents in lyophilized form for the synthesis of first-strand, full-length cDNA from purified poly(A)$^+$ or total RNA. The first-strand reaction mix is aliquoted into plate wells or strip wells and then lyophilized, allowing for ambient temperature storage and handling, the addition of large sample volumes, and ease of reaction setup. To perform first-strand synthesis, simply add water, primers, and template, vortex to dissolve the pellet, and proceed with the reaction.

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.

Each pellet contains SuperScript™ III Reverse Transcriptase, a proprietary buffer system, $MgCl_2$, dNTPs, RNaseOUT™ Recombinant Ribonuclease Inhibitor, and stabilizers. The final concentration of dNTPs is 500 µM each and the final concentration of $MgCl_2$ is 5 mM.

SuperScript™ III Reverse Transcriptase is a version of M-MLV RT that has been engineered to have reduced 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.

A separate tube of Annealing Buffer is included in each kit for use in the initial template-primer annealing step. 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 2.

The final volume of each cDNA synthesis reaction, including primers, template, and water, is 20 µl.

Component	Amount
Cat. no. 18580-008	
12 × 8 strip wells, plate format	96 reactions
Annealing Buffer	2 × 50 µl
Cat. no. 18580-096	
96-well plate	96 reactions
Annealing Buffer	2 × 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
Micro-to-Midi Total RNA Purification System	50 rxns	12183-018
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	

Quality Control

A minimum of 40 ng of a 1181-bp RT-PCR product was obtained from 10 pg of total HeLa RNA and human GAPDH primers. A minimum of 200 ng of a 5.6-kb RT-PCR product was obtained from 100 ng of total HeLa RNA and human DNCH primers.

This product is distributed for laboratory research use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Invitrogen TECH-LINE℠ 800 955 6288

Recommendations and Guidelines

Plate Formats

SuperScript™ III RTS First-Strand cDNA Synthesis reaction mix comes lyophilized in a 96-well microtiter plate or 12 × 8 strip wells in plate format.

96-Well Plate

The microtiter plate comes sealed. To open, peel away the foil seal to expose the wells you want to use and add the template/primer mix. Then reseal the wells for thermal cycling using the heat seal tape provided in each kit.

To seal wells for thermal cycling and subsequent storage:

1. Peel away the plastic backing to expose the sticky side of the heat seal tape.
2. Position the tape sticky-side down on the plate, so that all wells are covered. Press down gently and evenly to seal.

Important: Do not use the heat seal tape provided in the kit to cover wells containing unreconstituted pellets, because the pellets will stick to the tape. Wells containing pellets should remain covered with the original foil seal.

The plate can be cut into four sections of 24 wells each (8 wells × 3 rows). Each plate section can then be run in a separate reaction. To separate, carefully cut the foil seal and tabs connecting each section. Store any unused, foil-covered wells in the original resealable pouch with the desiccant pack to ensure dryness.

12 × 8 Strip Wells in Plate Format

Twelve 8-well strips come inserted in a 96-hole plate frame. Push up gently on the bottom of the wells to remove each strip from the frame.

Strip wells are sealed with snap-on strip-caps. Pull up gently on the end of a row of caps to unsnap them. To reseal the plates for thermal cycling and storage, snap the caps back into place. Store any unused strips in the original resealable pouch with the desiccant pack to ensure dryness.

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.
- To isolate total RNA, we recommend TRIzol® Reagent (Cat. Nos. 15596-026/-018) or the Micro-to-Midi Total RNA Purification System (Cat. no. 12183-018). 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 (Catalog 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.
- After preparation of the annealing mixture, transfer the mixture to a preheated thermal cycler.

Required Materials

- 0.2-ml thin-walled PCR tubes or PCR plate
- Thermal cycler, preheated to 65°C
- Optional: Ice
- RNase/DNase-free water
- Microcentrifuge
- Vortex mixer

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 Web site 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. Preheat the thermal cycler to 65°C. Combine the following in a separate 0.2-ml thin-walled PCR tube or plate well at room temperature or on ice:

Component	Amount
Template 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 20 µl

2. Incubate in a thermal cycler at 65°C for 5 minutes, and then cool down at room temperature or on ice for at least 1 minute. Collect the contents by brief centrifugation.
3. Remove the seal/caps from the RTS plate/strip wells. Add the RNA/primer mix to the pellet in a well at room temperature.
4. Seal the plate/strip wells (see page 2 for sealing instructions) and vortex for 5–10 seconds. **Note: Vortexing is crucial to ensure complete dissolution of the pellet.** Centrifuge briefly to collect the contents, and then 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
5. Terminate the reactions at 85°C for 5 minutes, and then cool down at room temperature or on ice.

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

Negative RT Control

To prepare a negative RT control reaction, first dissolve each pellet in 10 µl of RNase/DNase-free water and then heat the mixture to 90°C to inactivate the enzyme. Then prepare a mix of template, primer, and Annealing Buffer as described above at a final volume of 10 µl instead of 20 µl. Add the 10 µl of primer/template mix to the 10 µl of dissolved pellet and proceed with the reaction.

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 sure to include the Annealing Buffer when adding primers and template and vortex the reaction mixture to fully dissolve the pellet..
RNase contamination	Maintain aseptic conditions to prevent RNase contamination. RNaseOUT™ is included in the pellet 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.
	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

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.

Limited Use Label License No. 138: SuperScript™ III Reverse Transcriptase

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents owned by Invitrogen Corporation and claiming this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

Limited Use Label License No. 18: RNaseOUT™ Ribonuclease Inhibitor

This product is the subject of U.S. Patent No. 5,965,399 owned by Invitrogen Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

©2004 Invitrogen Corporation. All rights reserved.
For research use only. Not intended for any animal or human therapeutic or diagnostic use.