

SuperScript™ One-Step RT-PCR with Platinum® Taq

Cat. No. 10928-034

Size: 25 reactions

Cat. No. 10928-042

Size: 100 reactions

Store at -20°C

Description

The SuperScript™ One-Step RT-PCR with Platinum® Taq System is designed for the convenient, sensitive, and reproducible detection and analysis of RNA molecules by RT-PCR. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and target RNAs from either total RNA or mRNA. RT/ Platinum® Taq mix is a mixture of recombinant Taq DNA polymerase complexed with proprietary antibody that inhibits polymerase activity and SuperScript™ II Reverse Transcriptase. The activity of Taq DNA polymerase is blocked at ambient temperatures but is regained after the denaturation step in PCR cycling at 94°C. This reagent provides an automatic “hot start” for Taq DNA polymerase in PCR (1,2,3). Hot starts are typically used in PCR to increase sensitivity, specificity, and yield. The reaction conditions for SuperScript™ One-Step RT-PCR with Platinum® Taq have been optimized to support a wide range of RT-PCR applications including conventional end-point detection, semi-quantitative, and real-time quantitative procedures. All components necessary for RT-PCR are mixed in one tube, reverse transcription automatically follows PCR cycling without additional steps.

The system consists of two major components: SuperScript™ II RT/ Platinum® Taq Mix (RT/ Platinum® Taq Mix) and 2X Reaction Mix. The RT/ Platinum® Taq Mix contains a mixture of SuperScript™ II Reverse Transcriptase and Platinum® Taq DNA Polymerase for optimal cDNA synthesis and PCR amplification. The 2X Reaction Mix consists of a proprietary buffer system optimized for reverse transcription and PCR amplification, Mg²⁺ optimized for universal use, deoxyribonucleotide triphosphates and stabilizers. This convenient 2X format allows further addition of template and primer at any desired concentration. Two tubes of MgSO₄ are also included, 5 mM for conventional use, and 50 mM for quantitative protocols. Sufficient reagents are provided for 25 or 100 amplification reactions of 50 µl each.

Components

Store all components at -20°C

<u>Component</u>	<u>25-rxn kit</u>	<u>100-rxn kit</u>
RT/ Platinum® Taq Mix	25 µl	100 µl
2X Reaction Mix (a buffer containing 0.4 mM of each dNTP, 2.4 mM MgSO ₄)	1.25 ml	3 x 1.25 ml
5 mM Magnesium Sulfate	500 µl	500 µl
50 mM Magnesium Sulfate	1 ml	1 ml

Long RT-PCR and Quantitative RT-PCR

For amplification of long targets (up to 12.3 kb), use SuperScript™ One-Step RT-PCR for Long Templates.

For one-step quantitative RT-PCR, use the SuperScript™ III Platinum® One-Step Quantitative RT-PCR System.

Additional Products

The following products are also available from Invitrogen.

<u>Product</u>	<u>Amount</u>	<u>Catalog no.</u>
SuperScript™ One-Step RT-PCR for Long Templates	25 rxns	11922-010
	100 rxns	11922-028
SuperScript™ III Platinum® One-Step Quantitative RT-PCR System	100 rxns	11732-020
	500 rxns	11732-088
Micro-to-Midi Total RNA Purification System	50 rxns	12183-018
TRIzol® Reagent	100 ml	15596-026
	200 ml	15596-018
DNase I, Amplification Grade	100 units	18068-015
Custom Primers	to order, visit www.invitrogen.com	

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-LINESM 800 955 6288

Important Parameters**RNA**

- High quality intact RNA is essential for successful full-length cDNA synthesis.
- RNA should be devoid of any RNase contamination and aseptic conditions should be maintained.
- Recommended methods of total RNA isolation include the Micro-to-Midi Total RNA Purification System (Catalog no. 12183-018) and TRIzol[®] Reagent (Catalog no. 15596-026) (4, 5). Oligo(dT)-selection for poly(A)⁺ RNA is typically not necessary, although incorporating this step may improve the yield of specific cDNAs.

Primers

- Gene specific primers (GSP) are recommended. Use of oligo(dT) or random primers are not recommended as they result in generation of non-specific products in the one-step procedure and the amount of RT-PCR product may be reduced.
- A final primer concentration of 0.2 μ M for each primer is generally optimal. However, for best results, a primer titration using 0.15–0.5 μ M is recommended.
- Design primers that anneal to sequence in exons on both sides of an intron or exon/exon boundary of the mRNA to allow differentiation between amplification of cDNA and potential contaminating genomic DNA.
- Primers should not be self-complementary or complementary to each other at the 3' ends.

Magnesium

- 1.2 mM final concentration of magnesium in the reaction mix works well for most targets (7). If needed, the magnesium concentration can further be optimized (usually between 1.2–2 mM) with the 5 mM MgSO₄ solution provided.

DNTPs

- 200 μ M dNTP concentration is optimal for most RT-PCR reactions.

Recommendations and Tips

- Keep all components, reaction mixes, and samples on ice. After preparation of the samples, transfer them to a pre-heated thermal cycler (45–55°C, depending on the cDNA step temperature) and immediately start the RT-PCR amplification program.
- Efficient cDNA synthesis can be accomplished in a 15–30 min incubation at 45–55°C.
- SuperScript[™] II RT is inactivated, Platinum[®] *Taq* DNA polymerase is reactivated and the RNA/cDNA hybrid is denatured during the 2 min incubation at 94°C.
- The annealing temperature should be 10°C below the melting temperature of the primers used.
- The extension time varies with the size of the amplicon (approximately 1 min per 1 kb of amplicon).
- For all targets up to 3 kb, 1 μ l of RT/ Platinum[®] *Taq* Mix is sufficient.

Quality Control

The product is tested functionally using 10 pg of total HeLa RNA as the template for amplification of a 353-bp segment of β -actin mRNA (40 cycles). A minimum of 25 ng of the RT-PCR product was obtained under these conditions.

Protocol

1. Program the thermal cycler so that cDNA synthesis is followed immediately with PCR amplification automatically.

Note: The following cycling conditions were established using a DNA Thermal Cycler 9600 or 2400 (Perkin Elmer) and may have to be altered for other thermal cyclers. Efficient cDNA synthesis can be achieved in a 15-30 min incubation at 45-55°C. It is recommended that a 30-min incubation at 50°C be used as a general starting point. The optimal temperature for reverse transcription will depend on primer and target sequences. Remember that cycling conditions may have to be further optimized for different sequences. Annealing and extension steps are separate (three-step cycling).

A: cDNA synthesis and pre-denaturation	B: PCR amplification	C: Final extension-(optional)
Perform 1 cycle of: 45-55°C for 15-30 min 94°C for 2 min	Perform 35-40 cycles of: Denature, 94°C for 15 s Anneal, 55-60°C for 30 s Extend, 68-72°C for 1 min/kb	1 cycle of 72°C for 5-10 min For use in Perkin-Elmer Model 480 cycler, use 30 s denaturation instead of 15 s.

2. Add the following to the microcentrifuge tubes placed on ice. Reaction cocktails can be made when multiple reactions are being assembled.

<u>Components</u>	<u>Volume/50 µl</u>	<u>Final Concentration</u>
2X Reaction Mix	25 µl	1X
Template RNA	x µl	10 pg – 1 µg
Sense Primer (10 µM)	1 µl	0.2 µM
Anti-sense Primer (10 µM)	1 µl	0.2 µM
RT/ Platinum® <i>Taq</i> Mix	1 µl	—
Autoclaved distilled water	to 50 µl	—

Note: Absence of genomic DNA in RNA preparations can be verified by omitting the RT/ Platinum® *Taq* Mix and substituting 2 units of Platinum® *Taq* DNA polymerase in the reaction.

3. Gently mix and make sure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed. Depending on the thermal cycler used, overlay with silicone oil, if necessary.
4. Analyze the amplification product.

Troubleshooting Guide

Problem	Possible cause	Possible solution
No amplification product	<ul style="list-style-type: none"> • No cDNA synthesis (temperature too high) • RNase contamination • Not enough starting template RNA 	<ul style="list-style-type: none"> • For the cDNA synthesis step, incubate <55°C. • Maintain aseptic conditions; add RNase inhibitor. • Increase the concentration of template RNA; use 100 ng to 1 µg of total RNA.
	<ul style="list-style-type: none"> • RNA has been damaged or degraded • RT inhibitors are present in RNA 	<ul style="list-style-type: none"> • Replace RNA if necessary. • Remove inhibitors in the RNA preparation by an additional 70% ethanol wash. NOTE: Inhibitors of RT include SDS, EDTA, guanidium salts, formamide, sodium phosphate and spermidine (8,9).
	<ul style="list-style-type: none"> • Annealing temperature is too high 	<ul style="list-style-type: none"> • Decrease temperature as necessary.
	<ul style="list-style-type: none"> • Extension time is too short 	<ul style="list-style-type: none"> • Set extension time for at least 60 s per kb of target length.
	<ul style="list-style-type: none"> • Cycle number is too low 	<ul style="list-style-type: none"> • Increase cycle number.
Low specificity	<ul style="list-style-type: none"> • Reaction conditions not optimal 	<ul style="list-style-type: none"> • Optimize magnesium concentration. • Optimize the primer. • Optimize the annealing temperature and extension time. • Increase temperature of RT reaction to 50-55°C.
	<ul style="list-style-type: none"> • Oligo(dT) or random primers used for first strand synthesis 	<ul style="list-style-type: none"> • Use gene-specific primers.
Unexpected bands after electrophoresis	<ul style="list-style-type: none"> • RNA contamination with genomic DNA 	<ul style="list-style-type: none"> • Pre-treat RNA with DNase I.

References

1. Chou, Q., et al. (1992) *Nucl. Acids Res.*, 20, 1717.
2. Sharkey, D.J., et al. (1994) *BioTechnology*, 12, 506.
3. Westfall, B.A., et al. (1997) *Focus*[®], 19.3, 46.
4. Chomczynski, P and Sacchi, N. (1987) *Anal. Biochem.* 162, 156.
5. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., and Rutter, W.J. (1979) *Biochemistry* 18, 5294.
6. Simms, D. (1993) *Focus*[®] 15, 6.
7. Sitaraman K., Lee, E.H., and Rashtchian, A. (1997) *Focus*[®] 19, 43.
8. Berger, S.L. and Kimmel, A.R. (1987) *Methods in Enzymol.* 152, 316.
9. Gerard, G. F. (1994) *Focus*[®] 16, 102.
10. Higuchi, R., Dollinger, G., Walsh, P.S., and Griffith, R. (1992) *Biotechnology* 10, 413.
11. Higuchi, R., Fockler, C., Dollinger, G., and Watson, R. (1993) *Biotechnology* 11, 1026
12. Holland, P.M., Abramson, R.D., Watson, R., and Gelfand, D. H. (1991) *Proc. Natl. Acad. Sci. USA* 88, 7276.
13. Gibson, U. E. M., Heid, C. A., and Williams, P.M. (1996) *Genome Res.* 6, 995.
14. Heid, C. A., Stevens, J., Livak, K. J., and Williams, P. M. (1996) *Genome Res.* 6, 986.
15. Tyagi, S. and Kramer, F.R. (1996) *Nature Biotechnology* 14, 303.
16. Tyagi, S., Bratu, D.P., and Kramer, F.R. (1998) *Nature Biotechnology* 16, 49.
17. Kostrikis, L.G., Tyagi, S., Mhlanga, M.M., Ho, D.D., and Kramer, F.R. (1998) *Science* 279, 1228.
18. Higuchi, R., Fockler, C., Dollinger, G., and Watson, R. (1993) *Biotechnology* 11, 1026
19. Wittwer, C.T., Herrmann, M.G., Moss, A.A., and Rasmussen, R.P. (1997) *Biotechniques* 22,130.

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