invitrogen
by life technologies

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

Catalog Numbers	Size	Store at –30°C to –10°C
10928-034	25 reactions	
10928-042	100 reactions	

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Description

Use the SuperScript® One-Step RT-PCR with Platinum® *Taq* System for the convenient, sensitive, and reproducible detection and analysis of RNA molecules by RT-PCR. Components for both cDNA synthesis and PCR are combined in a single tube, using gene-specific primers and target RNAs from either total RNA or mRNA. Reverse transcription automatically follows PCR cycling without additional steps.

The system consists of two major components: 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. SuperScript® II RT is a modified version of Moloney Murine Leukemia Virus (M-MLV) RT, engineered to reduce RNase H activity and increase thermal stability (Kotewicz, 1985; Gerard, 1986). Platinum® *Taq* DNA polymerase is *Taq* DNA polymerase complexed with a proprietary antibody that inhibits polymerase activity at ambient temperatures. The antibody is denatured and polymerase activity is restored during the denaturation step in PCR cycling at 94°C, which provides an automatic "hot start" in PCR, increasing sensitivity, specificity, and yield (Chou, 1992; Sharkey, 1994; Westfall, 1997).

The 2X Reaction Mix consists of a proprietary buffer system optimized for reverse transcription and PCR amplification, Mg^{2+} 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 $MgSO_4$ (5 mM and 50 mM) are also included in the kit. Sufficient reagents are provided for 25 or 100 amplification reactions of 50 µL each.

Note: SuperScript® One-Step RT-PCR with Platinum® *Taq* has been optimized to support end-point detection. For real-time quantitative one-step RT-PCR, we recommend the SuperScript® III Platinum® One-Step Quantitative RT-PCR System. For amplification of long targets (up to 12.3 kb), use SuperScript® One-Step RT-PCR for Long Templates.

Contents

Store all components at –30°C to –10°C.

Component	25-rxn kit	100-rxn kit
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 mL	3 × 1 mL
5 mM Magnesium Sulfate	500 µL	500 µL
50 mM Magnesium Sulfate	1 mL	1 mL

Additional Products

The following products are also available from Life Technologies.

Product	Amount	Catalog no.
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
TRIzol® Reagent	100 mL	15596-026
	200 mL	15596-018
DNase I, Amplification Grade	100 units	18068-015
Custom Primers	to order, visit **www.lifetechnologies.com**	

For Research Use Only. Not for use in diagnostic procedures.

Important Parameters

RNA

- Use high quality intact RNA for successful full-length cDNA synthesis.
- Make certain that RNA is devoid of any RNase contamination and maintain aseptic conditions.
- We recommend isolating total RNA with TRIzol® Reagent (Catalog no. 15596-026) (Chomcyznski, 1987; Chirgwin, 1979). Oligo(dT)-selection for poly(A)$^+$ RNA is typically not necessary, although incorporating this step may improve the yield of specific cDNAs.

Primers

- We recommend using gene specific primers (GSP). Use of oligo(dT) or random primers is not recommended, because they generate 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 µM for each primer is generally optimal. However, for best results, a primer titration using 0.15–0.5 µM 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 (Sitaraman, 1997). If needed, the magnesium concentration can further be optimized (usually between 1.2–2 mM) with the 5 mM $MgSO_4$ solution provided.

DNTPs

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

Recommendations and Tips

- Keep all components, reaction mixes, and samples on ice. After preparing 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 minute 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 minute 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 minute per 1 kb of amplicon).
- For all targets up to 3 kb, 1 µL of RT/ Platinum® *Taq* Mix is sufficient.

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 minute incubation at 45–55°C. We recommend starting with a 30-minute incubation at 50°C 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 minutes 94°C for 2 minutes	Perform 35–40 cycles of: Denature, 94°C for 15 seconds Anneal, 55–60°C for 30 seconds Extend, 68–72°C for 1 minute/kb	1 cycle of 72°C for 5–10 minutes For Perkin-Elmer Model 480 cycler, use 30 second denaturation instead of 15 seconds.

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

Components	Volume/50 µL	Final Concentration
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® *Taq* Mix	1 µL	—
Autoclaved distilled water	to 50 µL	—

   Note: Verify the absence of genomic DNA in RNA preparations 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	No cDNA synthesis (temperature too high)	For the cDNA synthesis step, incubate <55°C.
	RNase contamination	Maintain aseptic conditions; add RNase inhibitor.
	Not enough starting template RNA	Increase the concentration of template RNA; use 100 ng–1 µg of total RNA.
	RNA has been damaged or degraded	Replace RNA if necessary.
	RT inhibitors are present in RNA	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 (Berger, 1987; Gerard, 1994).
	Annealing temperature is too high	Decrease temperature as necessary.
	Extension time is too short	Set extension time for at least 60 s per kb of target length.
	Cycle number is too low	Increase cycle number.
Low specificity	Reaction conditions not optimal	Optimize magnesium concentration.
		Optimize the primer.
		Optimize the annealing temperature and extension time.
		Increase temperature of RT reaction to 50–55°C.
	Oligo(dT) or random primers used for first strand synthesis	Use gene-specific primers.
Unexpected bands after electrophoresis	RNA contamination with genomic DNA	Pre-treat RNA with DNase I.

References

Berger, S.L.and Kimmel, A.R. (1987) *Methods in Enzymol. 152*, 316.
Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., and Rutter, W.J. (1979) *Biochemistry 18*, 5294.
Chomczynski, P and Sacchi, N. (1987) *Anal. Biochem. 162*, 156.
Chou, Q., et al. (1992) *Nucl. Acids Res.*, *20*, 1717.
Gerard, G. F. (1994) *Focus® 16*, 102.
Gerard, G.F., D'Alessio, J.M., Kotewicz, M.L., and Noon, M.C. (1986) *DNA 5*, 271.
Gibson, U. E. M., Heid, C. A., and Williams, P.M. (1996) *Genome Res. 6*, 995.
Heid, C. A., Stevens, J., Livak, K. J., and Williams, P. M. (1996) *Genome Res. 6*, 986.
Higuchi, R., Dollinger, G., Walsh, P.S., and Griffith, R. (1992) *Biotechnology 10*, 413.
Higuchi, R., Fockler, C., Dollinger, G., and Watson, R. (1993) *Biotechnology 11*, 1026
Higuchi, R., Fockler, C., Dollinger, G., and Watson, R. (1993) *Biotechnology 11*, 1026
Holland, P.M., Abramson, R.D., Watson, R., and Gelfand, D. H. (1991) *Proc. Natl. Acad. Sci. USA 88*, 7276.
Kotewicz, M.L., D'Alessio, J.M., Driftmier, K.M., Blodgett, K.P., and Gerard, G.F. (1985) *Gene 35*, 249.
Kostrikis, L.G., Tyagi, S., Mhlanga, M.M., Ho, D.D., and Kramer, F.R. (1998) *Science 279*, 1228.
Sharkey, D.J., et al. (1994) *BioTechnology*, *12*, 506.
Simms, D. (1993) *Focus® 15*, 6.
Sitaraman K., Lee, E.H., and Rashtchian, A. (1997) *Focus® 19*, 43.
Tyagi, S., Bratu, D.P., and Kramer, F.R. (1998) *Nature Biotechnology 16*, 49.
Tyagi, S. and Kramer, F.R. (1996) *Nature Biotechnology 14*, 303.
Westfall, B.A., et al. (1997) *Focus®*, *19.3*, 46.
Wittwer, C.T., Herrmann, M.G., Moss, A.A., and Rasmussen, R.P. (1997*) Biotechniques 22*,130.

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