

Platinum[®] Quantitative RT-PCR ThermoScript[™] One-Step System

Cat. No. 11731-015
Cat. No. 11731-023

Size: 100 reactions
Size: 500 reactions
Store at -20°C

Description

The Platinum[®] Quantitative RT-PCR ThermoScript[™] One-Step System is a one-step real-time quantitative RT-PCR (qRT-PCR) system that is designed for the convenient, sensitive, and reproducible detection and quantification of RNA molecules. This system combines ThermoScript[™] Plus Reverse Transcriptase (RT) and Platinum[®] *Taq* DNA Polymerase in a single enzyme mix. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA. Reagents are provided for 100 or 500 amplification reactions of 50 µl each.

The system enables highly sensitive detection from as few as 10 copies of RNA template, with a broad dynamic range that supports accurate quantification of high-copy mRNA at up to 1 µg of total RNA.

- **ThermoScript[™] Plus RT** is an engineered mutant of avian retroviral reverse transcriptase that has increased thermostability compared to other RTs. Maximum activity for ThermoScript[™] Plus RT occurs between 45°C and 50°C; however, it supports cDNA synthesis up to 70°C.
- **Platinum[®] *Taq* DNA polymerase** is recombinant *Taq* DNA polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures (1, 2). Activity is restored after the denaturation step in PCR cycling, providing an automatic “hot start” in PCR for increased sensitivity, specificity, and yield.
- **2X ThermoScript[™] Reaction Mix** consists of a proprietary buffer system optimized for reverse transcription and PCR amplification, 6 mM MgSO₄ optimized for universal use, 0.4 mM of each dNTP, and stabilizers. This convenient 2X format allows further addition of template and primer at any desired concentration.

Reaction conditions for the system have been optimized to support both end-point RT-PCR and qRT-PCR. In qRT-PCR, the mix has been specifically formulated to provide optimal performance using fluorogenic probe-based detection technologies, such as TaqMan[®] probes (3–9). For one-step qRT-PCR using LUX[™] Primers, we recommend the SuperScript[®] III Platinum[®] One-Step Quantitative RT-PCR System. For one-step qRT-PCR using SYBR[®] Green I dye, we recommend the SuperScript[®] III Platinum[®] SYBR[®] Green One-Step qRT-PCR System. See **Additional Products**, below.

<u>Component</u>	<u>100-Rxn Kit</u>	<u>500-Rxn Kit</u>
ThermoScript [™] Plus/Platinum [®] <i>Taq</i> Mix	100 µl	500 µl
2X ThermoScript [™] Reaction Mix	2 × 1.25 ml	12.5 ml
50 mM Magnesium Sulfate	1 ml	2 × 1 ml
ROX Reference Dye	100 µl	500 µl

Storage

Store components at -20°C. Stability can be extended by storage at -80°C. **ROX Reference Dye must be stored in the dark.**

Additional Products

The following products are also available from Invitrogen.

<u>Product</u>	<u>Amount</u>	<u>Catalog no.</u>
SuperScript [®] III Platinum [®] One-Step Quantitative RT-PCR System	100 rxns	11732-020
	500 rxns	11732-088
SuperScript [®] III Platinum [®] SYBR [®] Green One-Step qPCR System	100 rxns	11736-051
	500 rxns	11736-059
PureLink [™] Micro-to-Midi [™] Total RNA Purification System	50 rxns	12183-018
TRIzol [®] Reagent	100 ml	15596-026
	200 ml	15596-018
RNaseOUT [™] Recombinant Ribonuclease Inhibitor	5000 units	10777-019
DNase I, Amplification Grade	100 units	18068-015

Recommendations and Guidelines for Quantitative RT-PCR

Template

Starting material can range from 1 pg to 1 µg of purified total RNA. If you are using purified mRNA, the amount of template may be reduced to as low as 0.5 pg.

RNA should be free of RNase contamination and aseptic conditions should be maintained. RNA may be treated with DNase I (Catalog no. 18068-015) to remove any contaminating genomic DNA.

Optional: An RNase inhibitor such as RNaseOUT™ (Cat. no. 10777-019) may be added to the reaction after the 2X Reaction Mix to safeguard against degradation of target RNA due to ribonuclease contamination.

Isolating Total RNA

To isolate total RNA, we recommend the PureLink™ Micro-to-Midi™ Total RNA Purification System (Cat. no. 12183-018), TRIzol® Reagent (Cat. nos. 15596-026 and 15596-018), or the PureLink™ 96 Total RNA Purification Kit for high-throughput applications (Cat. no. 12173-011). Isolation of mRNA from total RNA is typically not necessary, although incorporating this step may improve the yield of specific cDNAs.

Magnesium Concentration

The 2X ThermoScript™ Reaction Mix includes magnesium at a final concentration of 3 mM. This works well for most targets; however, the optimal concentration may range from 3 to 6 mM. If necessary, use the separate tube of 50-mM magnesium sulfate to increase the magnesium concentration. Use the following table to determine the amount of MgSO₄ to add to achieve the specified concentration (in a 50-µl PCR with 25 µl of 2X Reaction Mix):

For a Final MgSO ₄ Conc. of	Add 50-mM MgSO ₄ (per 50-µl Rxn)
4.0 mM	1 µl
5.0 mM	2 µl
6.0 mM	3 µl

Decrease the amount of water in the reaction accordingly.

ROX Reference Dye

ROX Reference Dye can be included in the reaction to normalize the fluorescent reporter signal, for instruments that are compatible with that option. ROX is supplied at a 25 µM concentration, and is composed of a glycine conjugate of 5-carboxy-X-rhodamine, succinimidyl ester in 20 mM Tris-HCl (pH 8.4), 0.1 mM EDTA, and 0.01% Tween® 20. Use the following table to determine the amount of ROX to use with a particular instrument:

Instrument	Amount of ROX per 50-µl reaction	Final ROX Concentration
ABI 7000, 7300 7700, 7900HT	1.0 µl	500 nM
ABI 7500; Stratagene Mx3000™, Mx3005P™, and Mx4000™	0.1 µl*	50 nM

*To accurately pipet 0.1 µl per reaction, we recommend that you dilute ROX 1:10 immediately before use and use 1 µl of the dilution.

Note that some TaqMan® probes utilize a TAMRA quencher, which can be used as a reference dye for normalization of data. This technique is only valid for an RT-PCR containing a single TaqMan® probe, and should not be used in multiplex applications.

Instrument Guidelines

This kit can be used with a variety of real-time instruments, including but not limited to the ABI PRISM® 7000, 7700, and 7900HT; the ABI 7300 and 7500 Real-Time PCR Systems; the ABI GeneAmp® 5700; the Bio-Rad iCycler™; the Stratagene Mx3000P®, Mx3005P™, and Mx4000®; the Corbett Research Rotor-Gene™; the MJ Research DNA Engine Opticon™, Opticon® 2, and Chromo 4™ Real-Time Detector; and the Cepheid Smart Cycler®. For instrument-specific guidelines, visit www.invitrogen.com/qpcr. Optimal cycling conditions will vary with different instruments.

Primers

Gene-specific primers (GSPs) are required. A final concentration of 200 nM per primer is effective for most reactions. Doubling the amount of reverse primer (to 400 nM) may improve the performance of certain reactions. Optimal results may require a primer titration between 100 and 500 nM.

Design primers that anneal to exons on both sides of an intron or within the exon/exon boundary of the mRNA to allow differentiation between amplification of cDNA and potential contaminating genomic DNA.

For multiplex applications, limit the amount of primer for the reference gene (e.g., β-actin or GAPDH) to avoid competition between amplification of the reference gene and sample gene. In general, the final concentration of the reference gene primer should be between 25 and 100 nM. However, a primer titration is recommended for optimal results.

Dual-Labeled Probes

- The optimal concentration of probe may vary between 50 and 800 nM. A recommended starting concentration is 100 nM.
- The probe sequence should be free of secondary structure and should not hybridize to itself or to primer 3' ends.
- For multiplex RT-PCR applications, the concentration of each probe may need to be adjusted independently to obtain optimal fluorescent signals. The amount of probe for the reference gene, such as β-actin or GAPDH, should be limited as described above for primers.

Reaction Setup and Conditions

- Keep all components, reaction mixes and samples on ice. After reaction assembly, transfer them to a thermal cycler pre-heated to the desired cDNA synthesis temperature to start the reaction.
- Efficient cDNA synthesis can be accomplished in a 15–30 min incubation at 50–70°C. The optimal temperature will vary for different primers and templates. 50°C is a good general starting point. For problematic templates, or to increase the specificity of cDNA priming, increase the cDNA synthesis temperature to 60°C. Use 70°C as a final option, because RNA template integrity in the presence of Mg²⁺ is short-lived at elevated temperatures.
- ThermoScript™ Plus RT is inactivated, the RNA/cDNA hybrid is denatured, and Platinum® Taq DNA polymerase is activated during the 5-minute incubation at 95°C.
- The annealing temperature should be 5–10°C below the melting temperature of the primers used.
- The extension time will vary with the size of the amplification product (approximately 1 minute per 1 kb).

PRISM® and GeneAmp® are registered trademarks of Applied Biosystems Corporation.

TaqMan® is a registered trademark of Roche Molecular Systems, Inc.

iCycler™, Mx3000P®, Mx3005P™, Mx4000®, Rotor-Gene™, DNA Engine Opticon™, Chromo 4™, and Smart Cycler® are trademarks or registered trademarks of their respective companies.

TRIzol® is a registered trademark of Molecular Research Center, Inc.

One-Step qRT-PCR Protocol

Follow the protocol below for one-step qRT-PCR using TaqMan® Probes on ABI real-time instruments. Note the lower amount of ROX Reference Dye required for the ABI 7500 systems (see page 2). This generic protocol may also be used for other real-time instruments.

For instrument-specific guidelines, visit www.invitrogen.com/qpcr. A standard 50-µl reaction size is provided; component volumes can be scaled as desired (e.g., scaled down to a 20-µl reaction volume for 384-well plates).

1. Program your real-time instrument to perform cDNA synthesis immediately followed by PCR amplification, as shown below. Optimal temperatures and incubation times may vary for different target sequences.

cDNA Synthesis: 50–70°C for 15–30 minutes (use 50°C for 30 minutes as a general starting point)

PCR: 95°C for 5 minutes (1 cycle)

35–45 cycles of:

95°C for 15 seconds

60–65°C for 60 seconds

2. Set up reactions **on ice**. Volumes for a single 50-µl reaction are listed below. For multiple reactions, prepare a master mix of common components, add the appropriate volume to each tube or plate well on ice, and then add the unique reaction components (e.g., template).

Note: Preparation of a master mix is essential in qRT-PCR to reduce pipetting errors.

Component	Single rxn
ThermoScript™ Plus/ Platinum® Taq Enzyme Mix	1 µl *
2X ThermoScript™ Reaction Mix	25 µl
Forward primer, 10 µM	1 µl
Reverse primer, 10 µM	1 µl
Fluorogenic probe, 10 µM	0.5 µl
ROX Reference Dye (optional)	1 µl/0.1 µl**
RNaseOUT™, 40 U/µl (optional)	1 µl
Template (1 pg to 1 µg total RNA)	≤ 10 µl
DEPC-treated water	to 50 µl

*To test for genomic DNA contamination of the RNA template, prepare a control reaction containing 2 units of Platinum® Taq DNA polymerase instead of the ThermoScript™ Plus/Platinum® Taq Mix.

**See the table on page 2 for the amount/concentration of ROX to use for your specific instrument.

3. Cap or seal the reaction tube/PCR plate, and gently mix. Make sure that all components are at the bottom of the tube/plate; centrifuge briefly if needed.
4. Place reactions in a preheated real-time instrument programmed as described above. Collect data and analyze results.

One-Step End-Point RT-PCR Protocol

Follow the protocol below for one-step end-point RT-PCR. The cycling conditions were established using a DNA Thermal Cycler 9600 or 2400 (Perkin-Elmer) and may need to be altered for other thermal cyclers. Optimal temperatures and cycling conditions will also depend on primer and target sequences. A standard 50-µl reaction size is provided; component volumes can be scaled as desired (e.g., scaled down to a 20-µl reaction volume for 384-well plates).

Cycling Program	Reaction Mix
cDNA synthesis: 50–70°C, 15–30 minutes ¹	<u>Component</u>
PCR: 95°C, 5 minutes (1 cycle)	ThermoScript™ Plus/ Platinum® Taq Enzyme Mix ³
35–40 cycles:	2X ThermoScript™ Reaction Mix
95°C, 15 seconds ²	Template RNA (10 pg–1 µg)
55–60°C, 30 seconds	Forward primer, 10 µM
68–72°C, 1 minute/kb	Reverse primer, 10 µM
	RNaseOUT™, 40 U/µl (optional)
	DEPC-treated water
	Per 50-µl Rxn
	1 µl
	25 µl
	x µl
	1 µl
	1 µl
	1 µl
	to 50 µl
¹ Use 60°C for 30 minutes as a general starting point.	³ To test for genomic DNA contamination of the RNA template, prepare a control reaction containing 2 units of Platinum® Taq DNA polymerase instead of the ThermoScript™ Plus/Platinum® Taq Mix.
² If using Perkin-Elmer Model 480, use 30 seconds denaturation instead of 15 seconds.	

1. Program the thermal cycler to perform cDNA synthesis immediately followed by PCR amplification, as specified above.
2. Set up reactions **on ice**. Add the reaction mix to a microcentrifuge tube or each well of a PCR plate placed on ice. When assembling multiple reactions, prepare a master mix of all components except template.
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. Place reactions in a preheated thermal cycler programmed as described above. Collect data and analyze results.

Quality Control

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

Troubleshooting Guide

Problem	Possible Cause	Probable Solution
No amplification product Relative fluorescent signal ≤ background or no template control	cDNA synthesis temperature too high, low priming efficiency RT or cDNA primer blocked by secondary structure RNA has been damaged or degraded RNase contamination Fluorescent probe not functional	Lower incubation temperature. Raise incubation temperature. Redesign primer(s). Replace RNA if necessary. Maintain aseptic conditions; add RNase inhibitor. Validate probe design and presence of fluorophore and quencher: Treat TaqMan [®] probe with DNase, and check for increase in fluorescence. Redesign and/or resynthesize probe if necessary.
Poor sensitivity	Not enough starting template RNA	Increase the concentration of template RNA; use 10 ng to 1 µg of total RNA.
Product detected at higher than expected cycle number	RNA has been damaged or degraded RNase contamination RT inhibitors are present in RNA Inefficient cDNA synthesis Inefficient PCR amplification	Replace RNA if necessary. Maintain aseptic conditions; add RNase inhibitor. Remove inhibitors in the RNA preparation by an additional 70% ethanol wash. Inhibitors of RT include SDS, EDTA, guanidium salts, formamide, sodium phosphate and spermidine (10, 11). Adjust cDNA synthesis temperature and/or primer design. Optimize PCR conditions: Adjust annealing temperature as necessary. Increase magnesium concentration. Redesign primers.
Higher than expected signal	Too much sample added to reactions	Decrease the concentration of template RNA.
Product detected at lower-than-expected cycle number, and/or positive signal from no-template controls	Template or PCR carry-over contamination	Isolate source of contamination and replace reagent(s). Use separate dedicated pipettors for reaction assembly and post-PCR analysis. Assemble reactions (except for target addition) in a DNA-free area. Use aerosol-resistant pipet tips or positive displacement pipettors.
Unexpected bands after electrophoresis	RNA contamination with genomic DNA Oligo dT or random primers used for 1st strand synthesis Low specificity in PCR	Pre-treat RNA with DNase I. Use gene-specific primers. Optimize PCR conditions as described above.

References

- Chou, Q., Russel, M., Birch, D., Raymond, J., and Bloch, W. (1992) *Nucl. Acids Res.* 20, 1717.
- Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., and Daiss, J.L. (1994) *BioTechnology* 12, 506.
- Tyagi, S. and Kramer, F.R. (1996) *Nature Biotechnology* 14, 303.
- Tyagi, S., Bratu, D.P., and Kramer, F.R. (1998) *Nature Biotechnology* 16, 49.
- Kostrikis, L.G., Tyagi, S., Mhlanga, M.M., Ho, D.D., and Kramer, F.R. (1998) *Science* 279, 1228.
- 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.
- Nazarenko, I.A., Bhatnagar, S.K., and Hohman, R.J. (1997) *Nucl. Acids Res.* 25, 2516.
- Wittwer, C.T., Herrmann, M.G., Moss, A.A., and Rasmussen, R.P. (1997) *Biotechniques* 22,130.
- Berger, S.L. and Kimmel, A.R. (1987) *Methods in Enzymol.* 152, 316.
- Gerard, G. F. (1994) *Focus*[®] 16, 102.

Limited Use Label License No. 1: Thermostable Polymerases

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224, 5,618,711, and 6,127,155. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Limited Use Label License No. 5: Invitrogen Technology

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. For products that are subject to multiple limited use label licenses, the terms of the most restrictive limited use label license shall control. Life Technologies Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Life Technologies Corporation which cover 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, Life Technologies is willing to accept return of the product with a full refund. For information about purchasing a license to use this product or the technology embedded in it for any use other than for research use please contact Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008; Phone (760) 603-7200 or e-mail: outlicensing@lifetech.com.

Limited Use Label License No. 14: Direct Inhibition by Anti-Polymerase Antibodies

Licensed to Life Technologies Corporation, under U.S. Patent Nos. 5,338,671; 5,587,287; and foreign equivalents for use in research only.

Limited Use Label License No. 274: 5' Nuclease Process

A license to perform the 5' nuclease process for research requires the use of a Licensed 5' Nuclease Kit (containing Licensed Probe), or the combination of an Authorized Core Kit plus Licensed Probe, or license rights that may be purchased from Applied Biosystems. This product is an Authorized Core Kit without Licensed Probe. Its purchase price includes a limited, non-transferable immunity from suit under U.S. Patents and corresponding patent claims outside the United States, owned by Roche Molecular Systems, Inc. or F. Hoffmann-La Roche Ltd ("Roche"), for using only this amount of the product in the practice of the 5' nuclease process solely for the purchaser's own internal research and development activities. This product is also an Authorized Core Kit for use with service sublicenses available from Applied Biosystems. This product conveys no rights under U.S. Patents Nos. 5,804,375, 6,214,979, 5,538,848, 5,723,591, 5,876,930, 6,030,787, or 6,258,569, or corresponding patent claims outside the United States, expressly, by implication, or by estoppel. No right under any other patent claims (such as apparatus or system claims) and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby granted expressly, by implication, or by estoppel. This product is for research purposes only. Diagnostic uses require a separate license from Roche. Further information regarding the 5' nuclease licensing program may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Limited Use Label License No. 316: One-Step RT-PCR

This Product is sold under license from bioMérieux under US Patent 5,654,143, US Patent 5,817,465, and/or any patent issuing from a reissue thereof and their foreign counterparts, and is for Research Use Only.

©2010 Life Technologies Corporation. All rights reserved.

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

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.