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# Platinum® *Tfi* One-Step qRT-PCR SuperMix

**Cat. no: 11771-200** **Size: 200 reactions (50 µl each)**

**Store at –20°C (non-frost-free)**

## Description

Platinum® *Tfi* One-Step qRT-PCR SuperMix provides components for one-step qRT-PCR in a convenient format that is compatible with fluorescent primer-based detection technologies, such as LUX™ Fluorogenic Primers. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA. The SuperMix offers comparable sensitivity and specificity to Platinum® *Taq*-based One-Step qRT-PCR mixes. In addition, you can obtain rights from Invitrogen to use Platinum® *Tfi* One-Step qRT-PCR SuperMix for non-research-use-only (RUO) applications. Consult our business development department at outlicensing@invitrogen.com for more information.

The kit includes two mixes. SuperScript™ III Enzyme Mix for One-Step qRT-PCR contains SuperScript® III Reverse Transcriptase and RNaseOUT™ Recombinant Ribonuclease Inhibitor. 2X Platinum® *Tfi* SuperMix includes Platinum® *Tfi* DNA Polymerase, $MgCl_2$, dNTPs (with dUTP instead of dTTP), heat-labile uracil DNA glycosylase (UDG), and stabilizers.

Platinum® *Tfi* Exo(–) DNA Polymerase is recombinant *Tfi* Exo(–) DNA polymerase plus a proprietary antibody mix that inhibits activity at room temperature, for an automatic hot start in qPCR. The enzyme has 5' → 3' polymerase activity, but lacks both 5' → 3' and 3' → 5' exonuclease activity (Shandilya et al., 2004). This unique one-step formulation includes a heat-labile form of UDG to help prevent reamplification of carryover PCR products between reactions (Lindahl *et al.*, 1977; Longo *et al.*, 1990). ROX Reference Dye is also provided to normalize the fluorescent signal between reactions for instruments that are compatible with this option.

| Component | Amount |
|---|---|
| 2X Platinum® *Tfi* SuperMix for One-Step qRT-PCR | 5 ml |
| SuperScript® III Enzyme Mix for One-Step Platinum® *Tfi* qRT-PCR | 250 µl |
| ROX Reference Dye (25 µM) | 500 µl |

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## General Guidelines and Recommendations for One-Step qRT-PCR

- When working with RNA, maintain aseptic conditions and use disposable, individually wrapped, sterile plasticware, aerosol resistant pipette tips, and RNase-free microcentrifuge tubes. RNase *Away*™ Reagent, a non-toxic solution, can be used to remove RNase contamination from surfaces (see **Additional Products**, page 4).
- This kit can be used with fluorescent primers, such as LUX™ Fluorogenic Primers (see **Additional Products**, page 4), and other qPCR detection technologies that do not require exonuclease activity in the polymerase. It is *not* suitable for use with fluorescent probes-based technologies, which require exonuclease activity.
- Starting material can range from 1 pg to 1 µg of purified total RNA. If you are starting with isolated mRNA, the amount of template may be as low as 0.5 pg. RNA should be free of RNase contamination and aseptic conditions should be maintained. RNA may be treated with amplification-grade DNase I to remove any contaminating DNA.
- To isolate total RNA, we recommend the PureLink™ Micro-to-Midi™ Total RNA Purification System, TRIzol® Reagent, or the PureLink™ 96 Total RNA Purification Kit (see **Additional Products**, page 4).
- Total RNA can be quantitated using the Quant-iT™ RNA Assay Kit or UV absorbance at 260 nm (see **Additional Products**, page 4)
- Reaction volumes can be scaled from 5 µl to 100 µl, depending on the instrument.
- For most templates, efficient cDNA synthesis can be accomplished in a 5-minute incubation at 50°C. For problematic templates, or to increase the specificity of cDNA priming, increase the cDNA synthesis temperature up to 60°C.
- Melting curve analysis may be used with fluorescent primers to identify the presence of primer dimers and analyze specificity.
- ROX Reference Dye is recommended for fluorescence normalization on Applied Biosystems® instruments, and is optional for Stratagene's Mx3000P™, Mx3005P™, and Mx4000®. It is not required on other instruments. Consult your instrument documentation for ROX use and concentrations.

## Basic Protocol

Use the protocol below as a general starting point for one-step qRT-PCR.

1. Program your real-time instrument as follows:

   30°C for 5-minute hold (UDG activation)
   50°C for 15-minute hold (cDNA synthesis)
   95°C for 2-minute hold
   40 cycles of:
      95°C for 15 seconds
      60°C for 1 minute

   Optional: Melting curve analysis: 55°C–95°C (refer to instrument manual for specific programming)

2. Set up reactions on ice. Volumes for a 20-µl reaction are provided; component volumes can be scaled as needed. For 384-well plates, we recommend a maximum reaction volume of 10 µl per well. **Always prepare a master mix of common components for multiple reactions.**

| | 20-µl rxn |
|---|---|
| 2X Platinum® *Tfi* SuperMix for One-Step qRT-PCR | 10 µl |
| Fluorescent primer mix (200 nM final conc. each primer) | X µl |
| ROX Reference Dye (25 µM)* | X µl |
| SuperScript® III Enzyme Mix for One-Step Platinum® *Tfi* qRT-PCR | 0.5 µl |
| Template RNA (*e.g.*, 1 pg–1 µg total RNA) | 5 µl |
| DEPC-treated water | to 20 µl |

   *Not required on all instruments. Final concentration is instrument-specific.

3. Prepare control reactions as follows:

   **No-RT controls:** To test for genomic DNA contamination of the RNA sample, do not add the SuperScript® III Enzyme Mix.

   **No-template controls:** To test for genomic DNA contamination of the enzyme/primer mixes, do not add template RNA.

4. Cap or seal each tube/plate and gently mix. Centrifuge briefly if needed to make sure that all components are at the bottom of the tube/well.

5. Place the tube in the real-time instrument and run the program from Step 1. After cycling, maintain the reaction at 4°C. **Optional:** Analyze the amplification products by agarose gel electrophoresis.

## 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.

## Additional Products

| Product | Amount | Catalog no. |
|---|---|---|
| TRIzol® Reagent | 100 ml | 15596-026 |
| | 200 ml | 15596-018 |
| PureLink™ Micro-to-Midi™ Total RNA Purification System | 50 rxns | 12183-018 |
| Quant-iT™ RNA Assay Kit | 1 kit | Q-33140 |
| LUX™ Fluorogenic Primers | visit www.invitrogen.com/lux | |
| RNase *Away*™ Reagent | 250 ml | 10328-011 |

## References


1. Lindahl, T., Ljungquist, S., Siegert, W., Nyberg, B., and Sperens, B. (1977) DNA N-glycosidases: properties of uracil-DNA glycosidase from Escherichia coli. *J. Biol. Chem., 252*, 3286-3294
2. Longo, M., Berninger, M., and Hartley, J. (1990) Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene, 93*, 125-128
3. Shandilya, H., Griffiths, K., Flynn, E. K., Astatke, M., Shih, P. J., Lee, J. E., Gerard, G. F., Gibbs, M. D., and Bergquist, P. L. (2004) Thermophilic bacterial DNA polymerases with reverse-transcriptase activity. *Extremophiles, 8*, 243-251