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Discover superior RT-PCR results more easily than ever

SuperScript IV UniPrime One-Step RT-PCR System

The Invitrogen™ SuperScript™ IV UniPrime™ One-Step RT-PCR System is the latest addition to the Invitrogen™ SuperScript™ product family. It combines Invitrogen™ SuperScript™ IV Reverse Transcriptase (RT) with the novel Invitrogen™ UniPrime™ RT-PCR Master Mix for ease of use and unmatched one-step RT-PCR performance.

Highlights

- **Simplified workflow**—universal primer annealing at 60°C decreases optimization time
- **Easy pipetting**—colored buffers for visual tracking of reaction setup and direct sample loading on gels
- **Enabled automation**—two-phase hot-start activation mechanism for room-temperature setup and benchtop stability for up to 24 hours
- **Superior performance**—high yields and reliable target detection even with challenging RNA samples

Two-phase hot-start activation mechanism explained

The innovative two-phase hot-start mechanism allows temporal separation of the activities of the reverse transcriptase and DNA polymerase, in order to deliver high specificity and yield in one-step RT-PCR.

Reaction setup

18–23°C

Reverse transcriptase and DNA polymerase remain inactive because of the hot-start mechanism preventing nonspecific activity.

First hot-start activation phase

45–60°C

Reverse transcriptase is activated and cDNA synthesis is initiated. DNA polymerase remains inactive to prevent any residual activity.

Second hot-start activation phase

98°C

DNA polymerase is activated and reverse transcriptase is simultaneously inactivated to allow highly efficient and specific DNA amplification following this step.

Superior sensitivity down to 0.01 pg RNA

The high sensitivity of the SuperScript IV UniPrime One-Step RT-PCR System enables detection of low-abundance targets and allows for one-step RT-PCR experiments even when RNA input is limited.

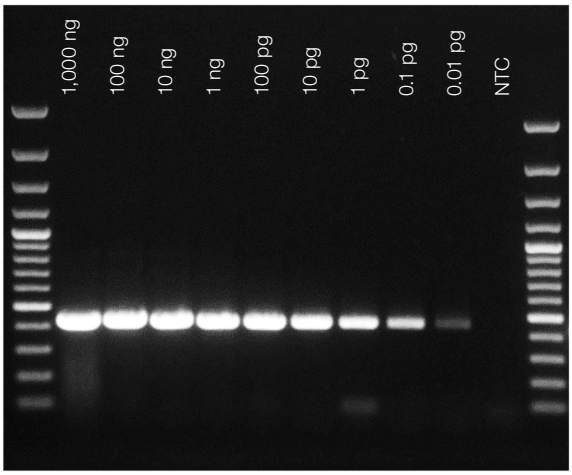

Figure 1. High sensitivity and reliable target detection from low amounts of input RNA. A 0.43 kb fragment was successfully amplified using serial dilution from 1,000 ng to 0.01 pg of Invitrogen™ Universal Human Reference RNA (UHRR) and the SuperScript IV UniPrime One-Step RT-PCR System. The molecular weight marker is the Thermo Scientific™ GeneRuler™ 100 bp Plus DNA Ladder, ready-to-use. NTC: no-template control.

Benchtop stability of preassembled reactions enables high-throughput applications

With its innovative two-phase hot-start activation mechanism, the SuperScript IV UniPrime One-Step RT-PCR System allows room-temperature reaction setup and stability of preassembled reactions for an extended length of time. For targets up to 3 kb, highly efficient and specific amplification is observed even after reactions are left for 24 hours at room temperature, and reactions can be stored for up to 4 hours for longer targets.

Avoid mistakes in RT-PCR runs with simplified reaction setup

RT-PCR assays using conventional reagents require specific protocols for amplification of each target due to varying primer annealing temperatures. With the SuperScript IV UniPrime One-Step RT-PCR System, the novel RT-PCR reaction buffer allows universal annealing at 60°C, helping minimize the optimization step and avoid mistakes (Figure 3).

Moreover, in the colored format, the SuperScript IV Reverse Transcriptase contains red tracking dye and the UniPrime RT-PCR Master Mix contains blue tracking dye. When the two solutions are mixed during reaction setup, the final reaction mix changes to purple, allowing you to visually track reaction setup and helping you avoid pipetting errors.

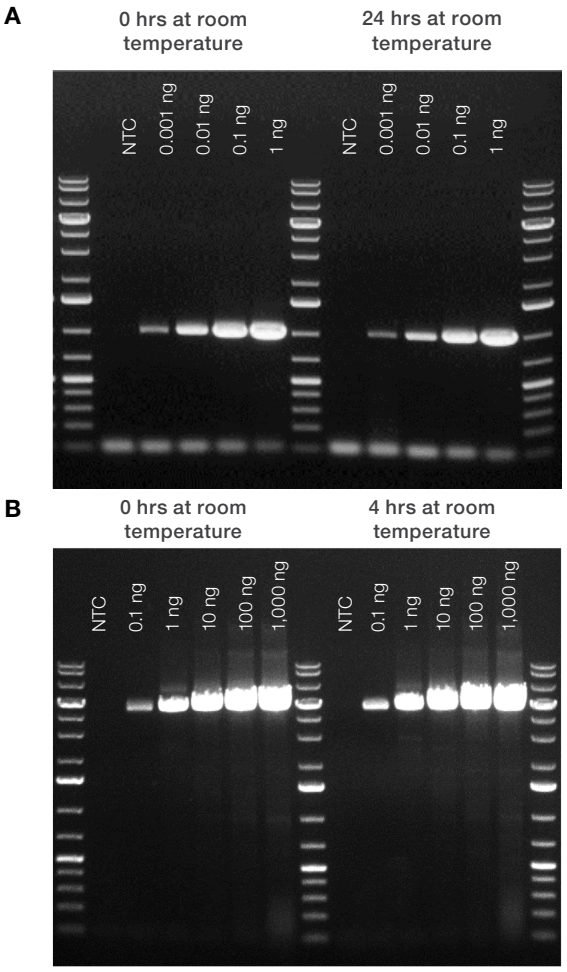

Figure 2. Extended stability at room temperature. Using the SuperScript IV UniPrime One-Step RT-PCR System, one-step RT-PCR reactions were assembled with **(A)** 1 kb RNA target from 0.001–1 ng UHRR and **(B)** 4.5 kb RNA target from 0.1–1,000 ng of UHRR. Assembled reactions were either immediately loaded on a thermal cycler or left at room temperature for **(A)** 24 hours and **(B)** 4 hours before cycling. Even after extended time at room temperature, highly efficient and specific target amplification was achieved. The molecular weight marker is the Thermo Scientific™ GeneRuler™ 1 kb Plus DNA Ladder, ready-to-use.

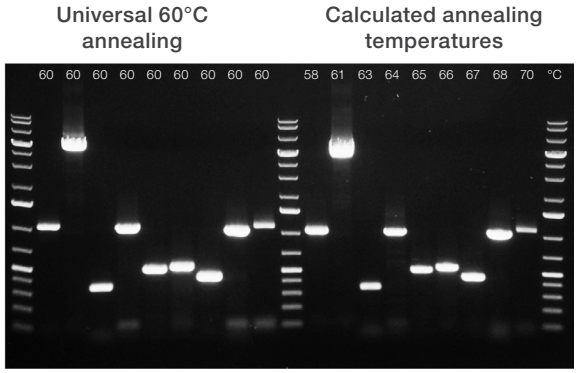

Figure 3. RT-PCR cycling under two annealing conditions. Nine targets with varying calculated annealing temperatures were amplified from 10 ng UHRR using a universal annealing temperature of 60°C (left), or the annealing temperatures calculated with the T_m calculator for Invitrogen™ Platinum™ SuperFi™ DNA Polymerase (right). The molecular weight marker is the GeneRuler 1 kb Plus DNA Ladder, ready-to-use.

Enhanced multiplex RT-PCR

With high specificity, high processivity, and a universal annealing feature, the SuperScript IV UniPrime One-Step RT-PCR System can successfully multiplex without the need for significant optimization steps.

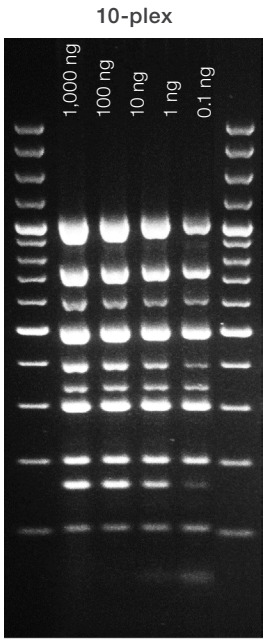

Figure 4. Multiplex RT-PCR from various RNA input amounts. Ten targets (101, 131, 199, 301, 346, 399, 498, 613, 734, and 1,006 bp) were amplified from 0.1–1,000 ng of UHRR. The molecular weight marker is the GeneRuler 100 bp Plus DNA Ladder, ready-to-use.

High processivity for better inhibitor tolerance, longer targets, and fast reactions

The processivity of a RT or DNA polymerase refers to the number of nucleotides incorporated in a single binding event of the enzyme. Therefore, highly processive enzymes can synthesize longer DNA strands in a shorter reaction time.

Enzyme processivity is also associated with affinity for the template. As such, RT and DNA polymerases with high processivity are resistant to common inhibitors that may have carried over from sample sources or purification steps (Figure 5).

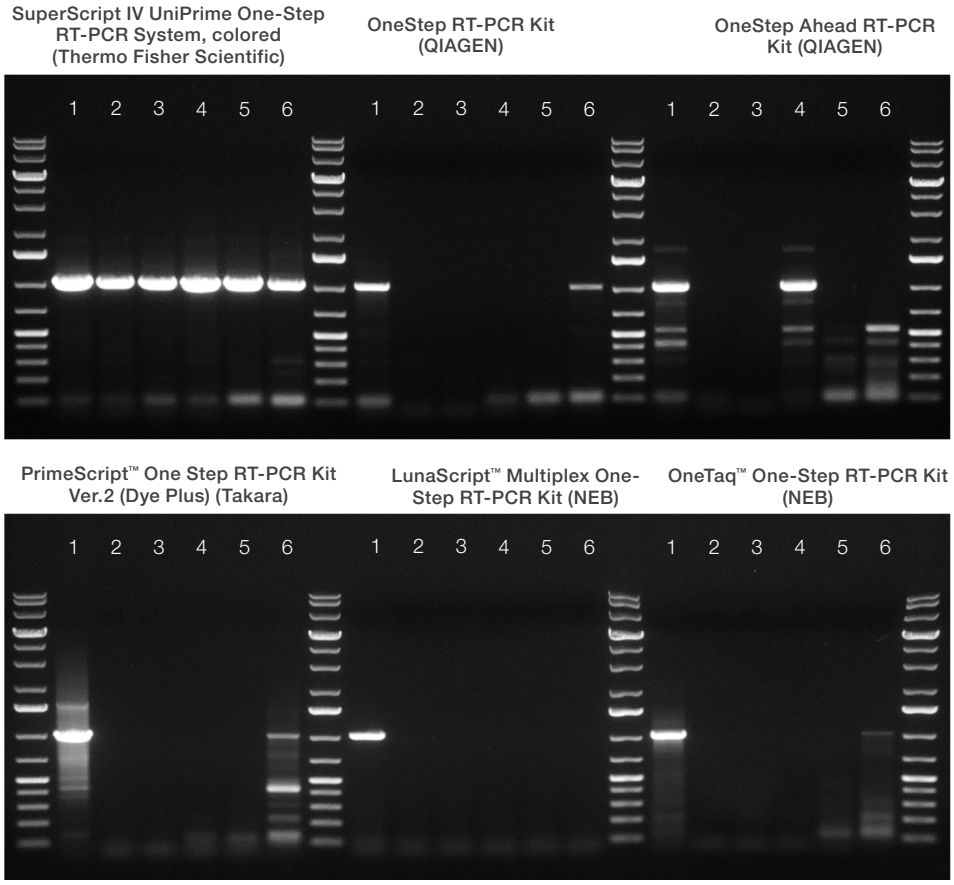

Figure 5. Resistance to inhibitors. Detection of a 1 kb RNA target from 100 ng UHRR using the SuperScript IV UniPrime One-Step RT-PCR System or other one-step RT-PCR kits in reaction mixtures containing: 1—no inhibitor, 2—xylan (2.5 µg/µL), 3—humic acid (15 ng/µL), 4—SDS (0.013%), 5—guanidinium thiocyanate (1.2%), and 6—LiCl (2.5 µg/µL). The enzymes in all kits except the SuperScript IV UniPrime One-Step RT-PCR System were inhibited by the indicated amounts of inhibitors. The molecular weight marker is the GeneRuler 1 kb Plus DNA Ladder, ready-to-use.

Broad range of RNA target lengths in significantly shorter times

Due to the high processivity of SuperScript IV Reverse Transcriptase and UniPrime RT-PCR Master Mix, the SuperScript IV UniPrime One-Step RT-PCR System enables detection of a broad range of target lengths (Figure 6). Full-length cDNA is synthesized in as short as a 10-minute RT reaction time, while the PCR step requires an extension time of only 30 sec/kb, resulting in one of the shortest protocols among available one-step RT-PCR kits (Figure 7).

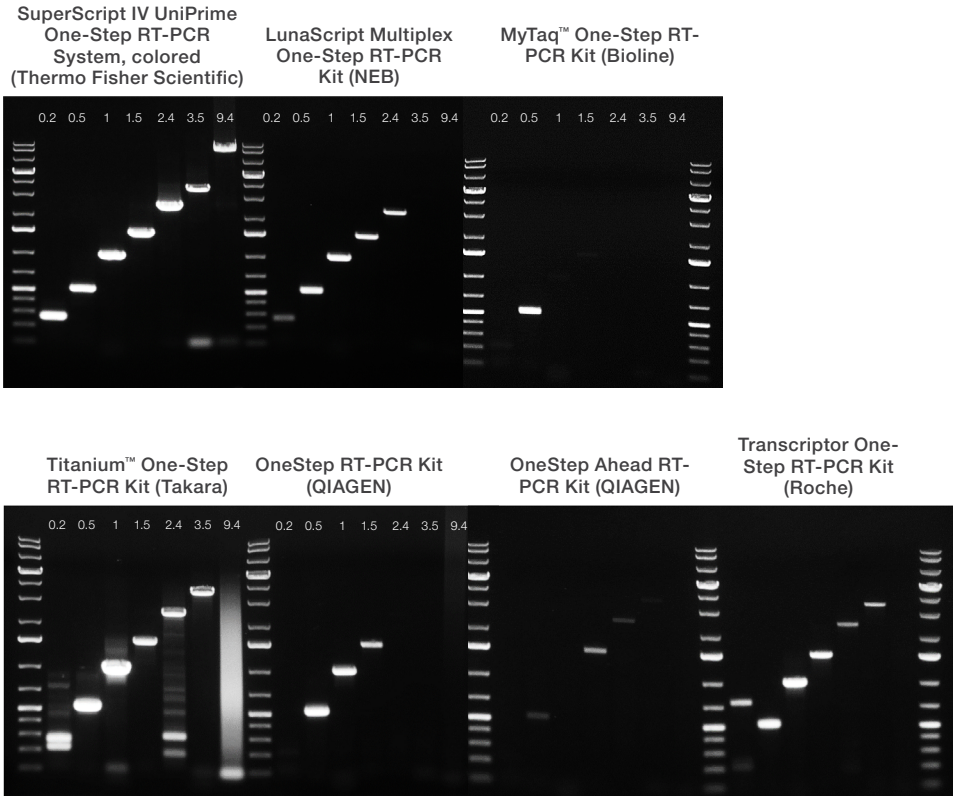

Figure 6. Versatility across a broad range of target lengths. Detection of RNA fragments ranging from 0.2 to 9.4 kb with the SuperScript IV UniPrime One-Step RT-PCR System and other one-step RT-PCR kits. The molecular weight marker is the GeneRuler 1 kb Plus DNA Ladder, ready-to-use.

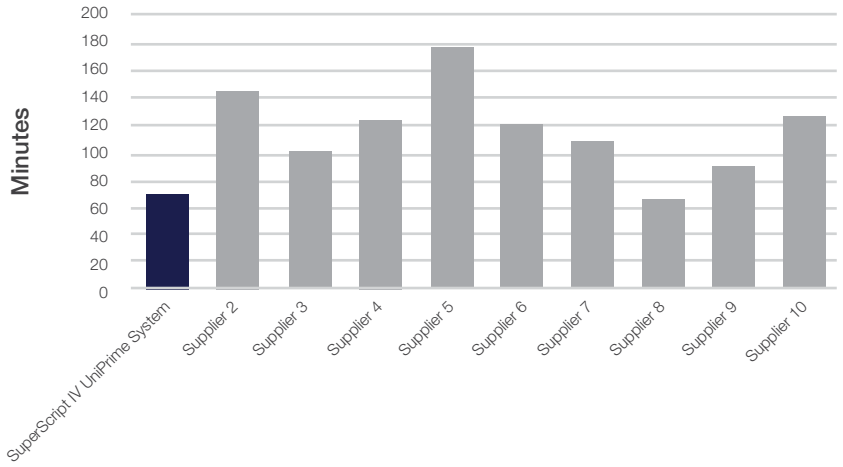

Figure 7. Total reaction times. A 1.5 kb target was amplified with the SuperScript IV UniPrime One-Step RT-PCR System and other one-step RT-PCR kits.

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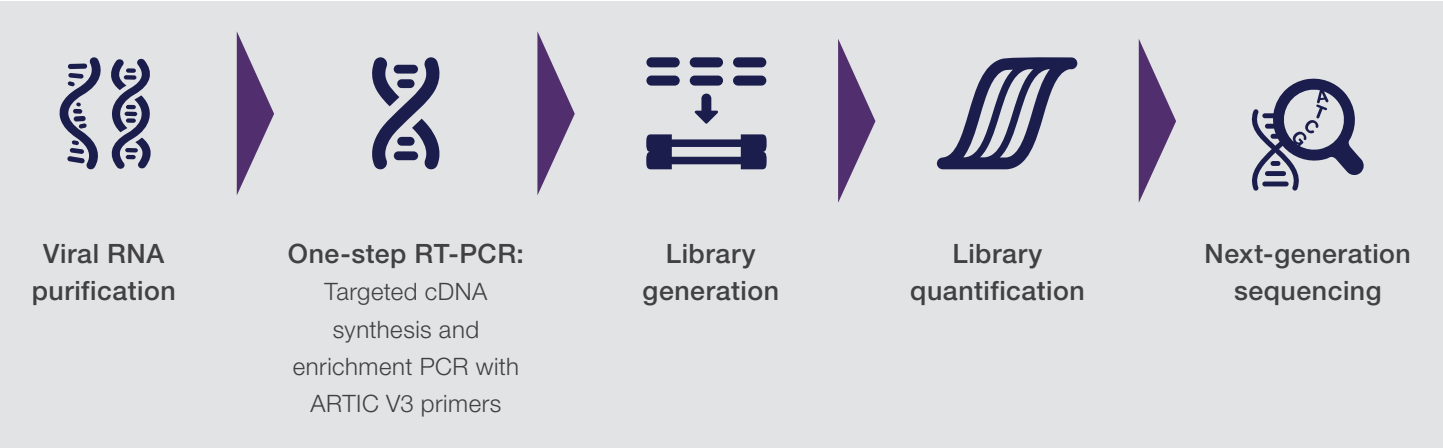
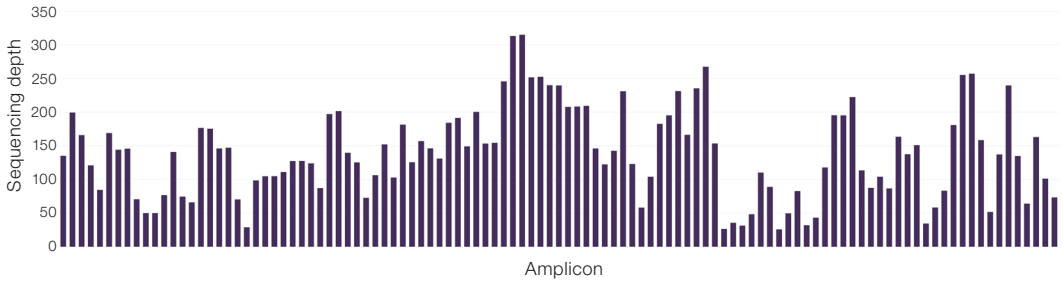
High multiplexing capacity enables next-generation sequencing (NGS) workflows

The high-level multiplexing capability of the SuperScript IV UniPrime One-Step RT-PCR System was successfully applied in a SARS-CoV-2 sequencing workflow, based on targeted amplification in which a primer panel from the ARTIC network [1] is used to generate amplicons to tile the entire SARS-CoV-2 genome.

NGS workflow using the SuperScript VI UniPrime One-Step RT-PCR System in a targeted amplification-based approach





Aligned reads	99.6%
Mean coverage	31.2x
Coverage >30x	89%

Figure 8. Average sequencing results of the whole SARS-CoV-2 genome. The whole SARS-CoV-2 genome (30 kb) was successfully covered with the sequencing of 109 amplicons from 24 SARS-CoV-2 clinical samples.

Ordering information

Description	Quantity	Cat. No.
SuperScript IV UniPrime One-Step RT PCR System	25 reactions	12-596-025
	100 reactions	12-596-100
	500 reactions	12-596-500
SuperScript IV UniPrime One-Step RT PCR System, Colored	25 reactions	12-597-025
	100 reactions	12-597-100
	500 reactions	12-597-500

1. ARTIC Network, artic.network/ncov-2019.

Learn more at **fishersci.com** and **fishersci.ca** to learn more.

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