Molecular diagnostics

Enhanced speed and precision—Lyo-ready SuperScript III Flash Reverse Transcriptase innovates in 1-step RT-qPCR applications

The Invitrogen™ Lyo-ready SuperScript™ III Flash Reverse Transcriptase joins the Invitrogen™ SuperScript™ product family as a new reverse transcriptase designed for 1-step RT-qPCR and point-of-care assay development. It is a novel variant of SuperScript III Reverse Transcriptase, engineered for enhanced speed, thermostability, and inhibitor tolerance. Lyo-ready SuperScript III Flash Reverse Transcriptase provides enhanced sensitivity of as low as 5 copies per reaction.

Highlights

- **Fast cDNA synthesis**—reaction time of 1 minute reduces time-to-results
- **Hot-start mechanism**—activity is blocked at ambient temperatures, enabling high-throughput applications, benchtop stability, and improved specificity of 1-step RT-qPCR
- **Thermostability**—reaction temperatures up to 70°C allow cDNA synthesis from complex RNA targets
- **High inhibitor tolerance**—system is less dependent on RNA sample purity
- **Commercial rights and customization**—enables product usage for commercial purposes
- **Excellent for 1-step RT-qPCR**—for a complete lyo-ready 1-step RT-qPCR solution, we recommend using Lyo-ready SuperScript III Flash Reverse Transcriptase together with Invitrogen™ Lyo-ready Platinum™ II *Taq* Hot-Start DNA Polymerase

Materials and methods

Reagents

Lyo-ready enzymes are provided in a glycerol-free format that helps ensure compatibility with microfluidics, air-drying, and lyophilization technologies. Lyo-ready enzymes retain characteristics of conventional enzymes, including reproducibility, sensitivity, and specificity required for diagnostic assays.

- **Lyo-ready SuperScript III Flash Reverse Transcriptase**—an engineered version of Moloney murine leukemia virus (MMLV) reverse transcriptase, designed especially for the one-step RT-qPCR application. The enzyme has increased reverse transcription (RT) synthesis speed and thermal stability. The hot-start mechanism ensures that the activity of the enzyme is blocked in ambient conditions, which provides high specificity without the need for additional time for enzyme reactivation.
- **Lyo-ready Platinum II *Taq* Hot-Start DNA Polymerase**—an engineered *Taq* DNA polymerase that shows increased tolerance to reaction inhibitors originating from sample materials or nucleic acid purification steps. Like the standard *Taq* DNA polymerase, it has both 5′ to 3′ polymerase and 5′ to 3′ exonuclease activities but lacks 3′ to 5′ exonuclease activity and can be used with hydrolysis probes. Due to an antibody-mediated hot-start mechanism, polymerase activity is blocked at ambient temperatures and restored after the initial denaturation step at 95°C. This automatic “hot start” increases sensitivity, specificity, and yield while allowing reaction assembly at room temperature.
- **5X Lyo-ready Platinum II PCR Buffer**—an optimized buffer designed to obtain exceptional performance from reverse transcriptase and polymerase combined in one-step RT-qPCR. The buffer’s composition helps ensure high reaction sensitivity, tolerance to reaction inhibitors, and compatibility with multiplexing and lyophilization.

Reaction setup and cycling conditions

Reaction conditions for viral RNA detection are summarized in Table 1.

The recommended cycling protocol is shown in Table 2.

Table 1. Reaction setup for 1-step RT-qPCR to detect RNA.

Component	Volume	Final concentration
Lyo-ready SuperScript III Flash Reverse Transcriptase	0.1 µL	0.3 U/µL
Lyo-ready Platinum II *Taq* DNA Polymerase	0.12 µL	0.12 U/µL
5X Lyo-ready Platinum II PCR Buffer	4.0 µL	1X
25 mM dNTP mix	0.48 µL	0.6 mM each
50 mM $MgCl_2$	4.0 µL	10 mM
10 µM forward primer*	0.6 µL each	0.3 µM each
10 µM reverse primer*	0.6 µL each	0.3 µM each
10 µM probe*	0.4 µL each	0.2 µM each
50 µM ROX™ reference dye**	0.02 µL	50 nM
Nuclease-free water	To 20 µL	–
RNA†	1 µL	Various

* Alternatively, 20X Applied Biosystems™ TaqMan™ Gene Expression Assays can be used at 1X final concentration.

** ROX or other reference dye is optional and may be used for reporter signal normalization. The concentration of reference dye should be chosen based on the qPCR instrument used.

† The volume of template RNA can be increased with a corresponding decrease in water in the reaction mix.

Table 2. Recommended cycling conditions for 1-step RT-qPCR using Lyo-ready SuperScript III Flash Reverse Transcriptase and Lyo-ready Platinum II *Taq* DNA Polymerase.

Step	Time	Temperature	No. of cycles
Reverse transcription	1 minute	60°C	1
Initial denaturation, DNA polymerase activation	2 minutes	95°C	
Denaturation	1 second*	95°C	40–45
Annealing and extension	15 seconds*	60°C	

* The time for these steps might need to be adjusted depending on the instrument used.

Comparison of reverse transcriptases using minimal time for the RT step

A comparison of reverse transcriptase performance using a minimal RT step time was carried out by detecting SARS-CoV-2 RNA at 20,000, 2,000, 200, and 20 copies per reaction. Results acquired using Lyo-ready SuperScript III Flash Reverse Transcriptase and Lyo-ready Platinum II *Taq* DNA Polymerase were compared to results acquired using other vendors' kits designed for one-step RT-qPCR.

Furthermore, to emphasize the effect of Lyo-ready SuperScript III Flash Reverse Transcriptase, two reverse transcriptases from other vendors were used together with Lyo-ready Platinum II *Taq* DNA Polymerase. In all cases, the duration of the RT step was set to 1 minute, while all other conditions were chosen according to manufacturers' recommendations. All reactions were performed in triplicate on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System.

One-step RT-qPCR without a separate RT step

To show that Lyo-ready SuperScript III Flash Reverse Transcriptase can perform RT without a separate RT step, synthetic SARS-CoV-2 RNA at 20,000, 2,000, 200, and 20 copies per reaction was amplified without an RT step using the cycling conditions in Table 3. Reactions were performed in triplicate on the QuantStudio 7 Flex Real-Time PCR System.

Table 3. Cycling conditions without an RT step.

Step	Time	Temperature	No. of cycles
Initial denaturation, DNA polymerase activation	2 minutes	95°C	1
Denaturation	1 second	95°C	40
Annealing and extension	15 seconds	60°C	

Benchtop stability of assembled reactions

The effectiveness of the hot-start mechanisms used by both enzymes was shown by incubating assembled reactions at room temperature for 24 hours. Reactions were assembled according to Table 1 and divided into two identical qPCR plates. Reactions were performed in triplicate and contained either SARS-CoV-2 RNA at 20,000, 2,000, 200, and 20 copies per reaction or universal human reference RNA at 100 pg or 10 ng per reaction. *GUSB* mRNA was targeted in the universal human reference RNA.

The run using the control qPCR plate was started immediately after assembly, on the QuantStudio 7 Flex Real-Time PCR System, while the second assembled qPCR plate was kept at room temperature for 24 hours before running it on the same real-time PCR System.

Temperature gradient of RT step

The thermostability of Lyo-ready SuperScript III Flash Reverse Transcriptase was shown by performing reactions on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System. Using the Applied Biosystems™ VeriFlex™ temperature control technology, a temperature gradient was set on one plate from 60°C to 70°C in 2.5°C increments, for a total of 5 RT temperatures tested (with two replicates for each temperature). Reactions contained either SARS-CoV-2 RNA at 2,000 copies per reaction or universal human reference RNA at 10 ng per reaction. *GUSB* mRNA was targeted in the universal human reference RNA.

Performance in the presence of inhibitors

A set of tests was conducted to evaluate the performance of Lyo-ready SuperScript III Flash Reverse Transcriptase in the presence of common PCR inhibitors that can appear after sample preparation steps. To test tolerance to inhibitors, the assembled reaction mix was spiked with either 10 µM hemin, 2% isopropanol, or 2.5 µL (12.5% of total reaction volume) universal transport medium (UTM). Control reactions without inhibitors were also performed. In all cases, reactions were performed in triplicate, and SARS-CoV-2 RNA at 2,000 copies per reaction or a *GUSB* target in 10 ng universal human reference RNA was the target of detection.

Detection of limited amounts of target

To evaluate the sensitivity and efficiency of Lyo-ready SuperScript III Flash Reverse Transcriptase, reactions were set up using synthetic SARS-CoV-2 RNA at 20,000, 2,000, 200, 20, and 5 copies per reaction. The QuantStudio 7 Flex Real-Time PCR System was used to perform reactions. Each reaction was performed with 5 replicates to reduce the possibility of artifacts arising during the preparation of dilutions.

Use of partially degraded samples

Degraded RNA was prepared to stress-test the ability of Lyo-ready SuperScript III Flash Reverse Transcriptase to amplify targets in low-quality RNA. Degraded RNA was prepared by incubating HeLa total RNA in $MgCl_2$-containing buffer at 95°C for 2–8 minutes. Afterward, RNA was purified to remove $MgCl_2$, and its RNA integrity number (RIN) was evaluated using an Agilent™ 2100 Bioanalyzer™ system. A *GAPDH* target was amplified from 100 pg or 10 ng of prepared degraded RNA (RIN 2.85– 7.15). RNA of good quality (RIN 9.75) was used as a control. The QuantStudio 7 Flex Real-Time PCR System was used to perform reactions, with 3 technical repeats for each.

Comparison of reverse transcriptases in detection of multiple targets

Four-plex one-step RT-qPCR assays were performed using a mixture of viral nucleic acids. Nucleic acids from parainfluenza virus, adenovirus, and chikungunya virus were purified from Vircell™ inactivated virus suspensions using the Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Cat. No. A48310). Synthetic SARS-CoV-2 RNA was acquired from Twist Biosciences.

Five-plex one-step RT-qPCR assays were performed to detect 5 mRNA targets in 100 ng universal human reference RNA. The 5 targets chosen for this reaction were *RPE*, *PPIA*, *IGFBP21*, *GUSB*, and *HPRT*.

Results acquired using Lyo-ready SuperScript III Flash Reverse Transcriptase and Lyo-ready Platinum II *Taq* DNA Polymerase were compared to results acquired using one-step RT-qPCR kits from other vendors. Furthermore, to evaluate the effect of only reverse transcriptase, two reverse transcriptases from other vendors were used together with Lyo-ready Platinum II *Taq* DNA Polymerase. All reactions were performed in triplicate using the QuantStudio 7 Flex Real-Time PCR System. In all cases, recommended conditions provided by the manufacturer, including reaction setup and cycling, were followed.

Results

Comparison of reverse transcriptases using minimal time for the RT step

Lyo-ready SuperScript III Flash Reverse Transcriptase reduces the overall time required to perform the 1-step RT-qPCR assay. This is especially beneficial when working with large sample sets or time-sensitive experiments, to obtain results quickly. Minimizing the duration of the RT step is also beneficial in high-throughput assays where numerous samples must be processed. With an RT step of only 1 minute, Lyo-ready SuperScript III Flash Reverse Transcriptase shows the lowest C_t values and maintains a linear response to input compared to stand-alone reverse transcriptases or ready-to-use master mixes from other vendors (Figure 1).

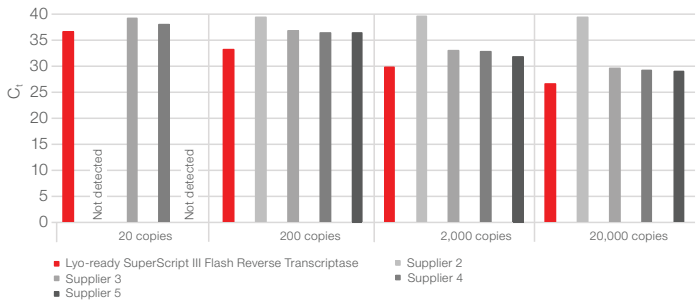

Figure 1. Comparison of reverse transcriptases using a 1-minute RT step and different amounts of RNA sample.

One-step RT-qPCR without a separate RT step

The RT step time could be eliminated if the analyzed RNA contains simply structured, highly expressed transcripts. Testing of Lyo-ready SuperScript III Flash Reverse Transcriptase in the 1-step RT-qPCR assay with an RT step time of 1 minute or without an RT step time shows a C_t difference of <2 cycles (Figure 2). This indicates that there is minimal variation in the amplification of the target gene between the two RT conditions.

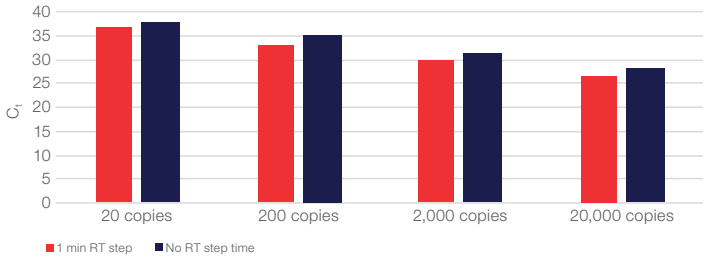

Figure 2. Impact of RT step time in 1-step RT-qPCR using SARS-CoV-2 RNA.

The findings suggest that the reverse transcriptase used in the 1-step RT-qPCR assay performs consistently well, regardless of whether a 1-minute RT step or no RT step time is used. This demonstrates the robustness and reliability of the assay in accurately detecting and quantifying gene expression.

Benchtop stability of assembled reactions

Lyo-ready SuperScript III Flash Reverse Transcriptase is kept inactive at ambient temperatures by a hot-start mechanism, which enables room temperature reaction setup and improves the specificity and consistency of RT-qPCR results. The results indicate that the hot-start mechanisms of the reverse transcriptase and DNA polymerase preserved the integrity and accuracy of the 1-step RT-qPCR reactions even after 24 hours at room temperature (Figure 3). This finding emphasizes compatibility with high-throughput applications, automation, and point-of-care assay development.

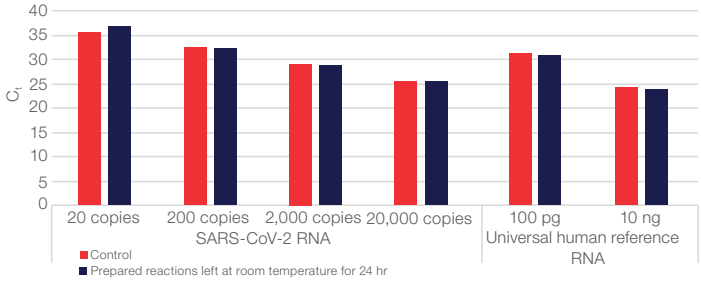

Figure 3. Benchtop stability of Lyo-ready SuperScript III Flash Reverse Transcriptase.

Temperature gradient of RT step

To overcome the challenge of detecting RNA with complex structures, Lyo-ready SuperScript III Flash Reverse Transcriptase provides a thermostability feature. Thermostability not only helps ensure the reliability of 1-step RT-qPCR but improves efficiency and specificity as well. It also helps ensure compatibility with various PCR cycling conditions and enables more straightforward adaptation to new assays. Lyo-ready SuperScript III Flash Reverse Transcriptase performs in reactions at temperatures up to 70°C (Figure 4) with no significant difference among various temperature points. Therefore, increased reaction temperatures do not have an impact on reverse transcriptase performance.

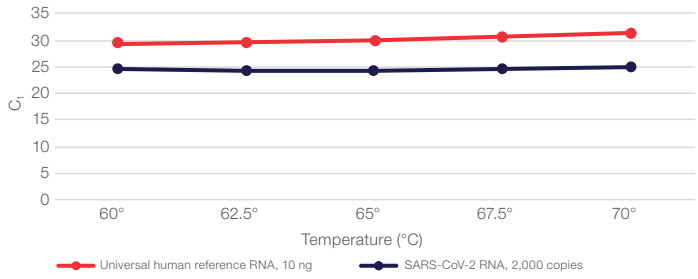

Figure 4. Thermostability of Lyo-ready SuperScript III Flash Reverse Transcriptase over a wide temperature range.

Performance in the presence of inhibitors

It can be challenging to obtain highly purified RNA, especially from complex biological samples. A 1-step RT-qPCR system with high tolerance to inhibitors may allow assay developers to utilize samples of suboptimal purity, thus potentially limiting the need for additional purification steps and preserving sample material. The results show that the most common inhibitors that might appear in the sample after sample preparation do not significantly impact 1-step RT-qPCR assay results, helping to assure reliable detection (Figure 5).

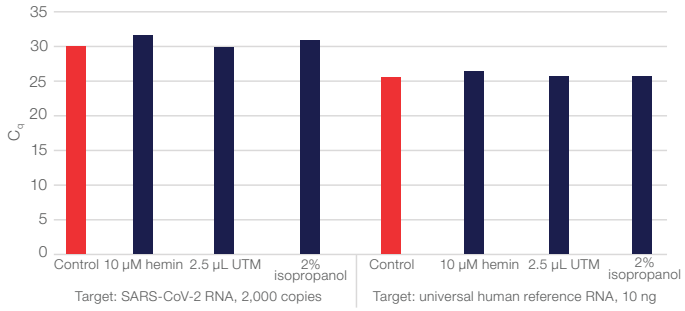

Figure 5. Inhibitor tolerance of Lyo-ready SuperScript III Flash Reverse Transcriptase.

Detection of limited amounts of target

Lyo-ready SuperScript III Flash Reverse Transcriptase enables sensitive detection from only 5 copies of RNA per reaction (Figure 6). Performing 1-step RT-qPCR with low amounts of RNA that meet or exceed market standards can enable detection of rare or low-expression targets without additional preamplification steps.

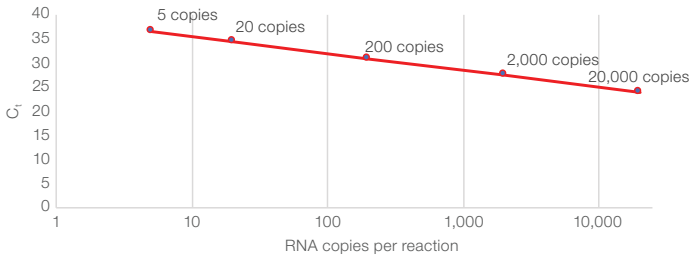

Figure 6. Sensitivity of Lyo-ready SuperScript III Flash Reverse Transcriptase.

Use of partially degraded samples

Lyo-ready SuperScript III Flash Reverse Transcriptase provides reliable detection even from low-quality RNA samples (Figure 7). This is a critical feature that helps ensure dependable results even when partial degradation of RNA can occur during sample collection, storage, or transport. It also allows for the maximum extraction of data because partially degraded RNA may still contain valuable information that can contribute to diagnostic outcomes.

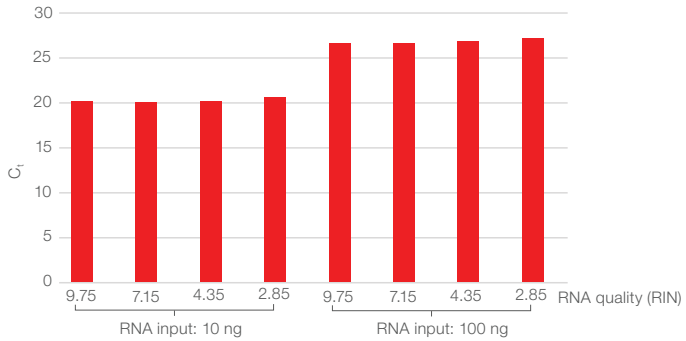

Figure 7. Partially degraded RNA test results.

Comparison of reverse transcriptases in detection of multiple targets

Instead of running individual reactions for each target, multiplexing enables the simultaneous detection of multiple targets, increasing experimental efficiency and reducing costs. Results obtained from 4-plex 1-step RT-qPCR assays show that reactions prepared with Lyo-ready SuperScript III Flash Reverse Transcriptase enable reliable detection of the 4 different targets (Figure 8). Moreover, the C_t values are lower than those obtained with stand-alone enzymes or ready-to-use master mixes from other vendors.

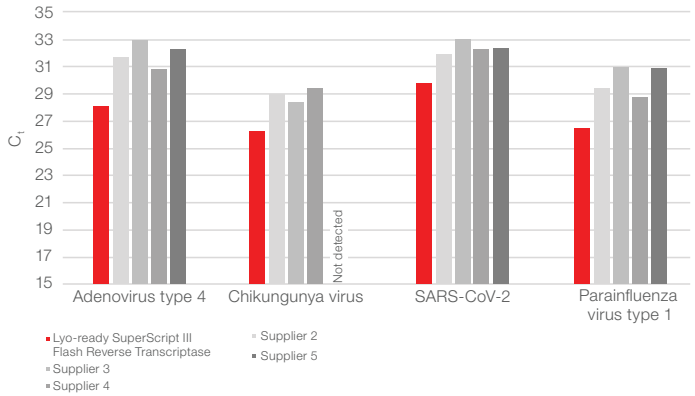

Figure 8. Multiplex detection of 4 different viral nucleic acids in a single reaction.

Lyo-ready SuperScript III Flash Reverse Transcriptase also enables the multiplexing of 5 different targets (Figure 9). Results obtained from 5-plex 1-step RT-qPCR assays show that reaction mixes prepared with Lyo-ready SuperScript III Flash Reverse Transcriptase enable reliable detection of the 5 different targets. The C_t values are again lower than those obtained with stand-alone enzymes or ready-to-use master mixes from other vendors.

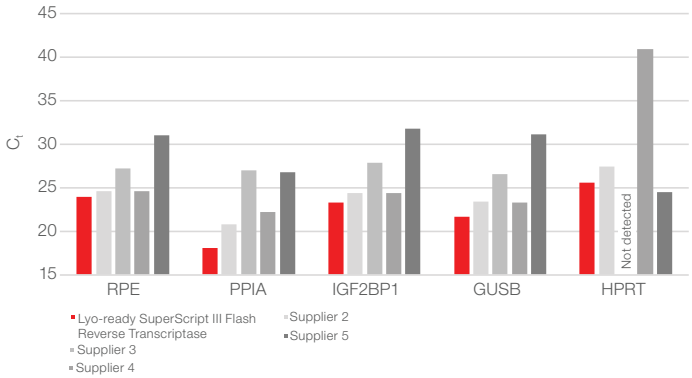

Figure 9. Multiplex detection of 5 different mRNA targets in a single reaction.

Conclusions

Lyo-ready SuperScript III Flash Reverse Transcriptase excels in 1-step RT-qPCR assays. It is capable of handling partially degraded RNA samples and is highly tolerant of inhibitors, sensitive, and thermostable up to 70°C. Its hot-start mechanism and robust performance with a minimal RT step time enable room temperature reaction setup and enhanced specificity and consistency in RT-qPCR results. Therefore, it offers a reliable and efficient solution for high-throughput and time-sensitive 1-step RT-qPCR applications.

Ordering information

Product	Quantity*	Cat. No.
Lyo-ready SuperScript III Flash Reverse Transcriptase	50 kU	EP212B6B001
	500 kU	EP212B6B002
	2,000 kU	EP212B6B003

* Quantity can be customized. To explore more customization capabilities, go to **thermofisher.com/mdx**.

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