Lyo-ready SuperScript Reverse Transcriptases: High-Performance Lyophilization-Compatible Enzymes for RT-PCR-based assays

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ABSTRACT

Nucleic acid detection assays in lyophilized (freeze-dried) format provide many advantages over conventional wet format in terms of higher stability and lower shipping costs. Conventional formulations of reverse transcriptases (RTs) used for RT-PCR contain up to 50% of glycerol, which serves as a cryoprotectant and stabilizes enzymes for extended periods of time. However, even at such minute concentrations as 0.5%, glycerol can interfere with the lyophilization process. Therefore, most of the conventional enzyme preparations are incompatible with lyophilization. Thermo Fisher Scientific has developed lyophilization-ready (lyo-ready) enzyme formulations for Invitrogen™ SuperScript™ IV (SSIV) and SuperScript III (SSIII) RTs that meet high-performance requirements of RT-PCR-based assays. The new lyo-ready RTs are formulated with less than 0.01% of glycerol and retain all inherent characteristics of enzyme preparations with glycerol, thus providing a powerful tool for development of sensitive and reproducible RT-PCR assays in dry formats.

MATERIALS AND METHODS

Selection of potential protein-stabilizing agents was carried out using a protein thermal shift assay. Stability of the selected formulations was tested in enzyme activity assays, and functionally in 2-step RT-qPCR. To assess lyo-compatibility, stand-alone lyo-ready enzymes were lyophilized using LyoBeta 25 (Telstar) and reconstituted in nuclease-free water to the initial volume. Performance of lyo-ready and glycerol formulations of RTs was compared in 2-step RT-qPCR.

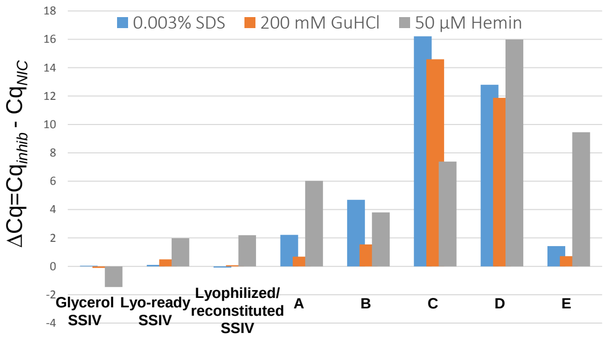
cDNA synthesis protocol for SSIII: 5 min at 25°C, 60 min at 50°C, 15 min at 70°C, and for SSIV: 10 min at 23°C, 10 min at 50°C, 10 min at 80°C.

RESULTS

Following figures demonstrate:

- Performance in the presence of inhibitors after lyophilization and reconstitution
- Linearity across a wide dynamic range using lyo-ready SSIV and SSIII in 2-step RT-qPCR
- Performance in 1-step RT-qPCR

Figure 1. Lyo-ready SuperScript RTs offer robust performance in the presence of inhibitors.

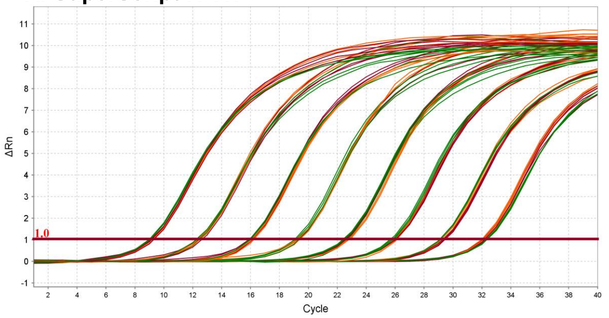


Cq_{NIC} – Cq of non inhibitor control
Cq_{inhib} – Cq in presence of inhibitor

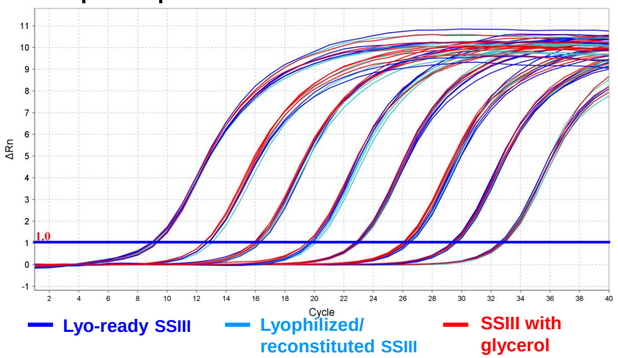
100 ng of T Cell Leukemia (Jurkat) Total RNA was used in 20 µL SSIV RT reactions containing RT inhibitors. Reactions with competitive RTs (A, B, C, D, E) were assembled according to manufacturers' protocols. Thermo Scientific™ Maxima™ Probe qPCR Master Mix with *PGK1* gene-specific primers was used for qPCR. cDNA composed 10% of the qPCR mixes.

Figure 2. Lyo-ready SuperScript RTs provide reliable performance across a wide dynamic range.

A. SuperScript IV RT

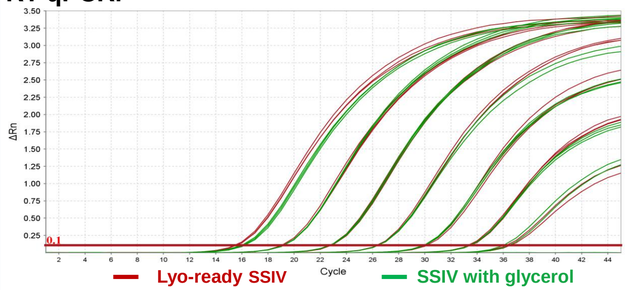


B. SuperScript III RT



1.25 ng to 0.125 fg of 1.3 kb GAPDH RNA transcript was used in 20 µL RT reactions with oligo(dT)$_{20}$ and random hexamers. Thermo Scientific™ Luminaris™ HiGreen qPCR Master Mix, low ROX with *GAPDH* gene-specific primers was used for qPCR. cDNA composed 10% of the qPCR mixes. Lyophilized and reconstituted lyo-ready SSIV and SSIII show performance equivalent to wet lyo-ready and glycerol-containing RTs. Lyo-ready enzymes show high linearity with $R^2 \geq 0.999$ and reaction efficiency of 100% for SSIV or 99% for SSIII across a wide dynamic range.

Figure 3. Lyo-ready SSIV compatibility in one-step RT-qPCR.



1 µg to 1 pg of Jurkat Total RNA was used in 1-step RT-qPCR with glycerol or lyo-ready SSIV, lyo-ready Invitrogen™ Platinum™ II *Taq* DNA polymerase, lyo-ready Thermo Scientific™ RiboLock™ RNase Inhibitor, and *B2M* gene-specific primers. 1-step RT-qPCR protocol: 15 min at 50°C, 3 min at 95°C, followed by 45 cycles of 5 s at 95°C and 15 s at 60°C. The reactions meet standard requirements for efficiency (90–110%) and linearity ($R^2 \geq 0.990$).

CONCLUSIONS

Thermo Fisher Scientific has developed stable, lyo-ready formats of reverse transcriptases with all of the same enzyme properties as conventional formulations and that are compatible with lyophilization.

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TRADEMARKS/LICENSING

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