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Spectacular is the new Super

SuperScript IV VILO Master Mix

Highest cDNA yields in the shortest time

ThermoFisher SCIENTIFIC

Invitrogen™ SuperScript™ IV VILO™ Master Mix is a cDNA reaction master mix designed for two-step quantitative reverse transcription PCR (RT-qPCR). It elevates the trusted VILO™ technology to the next level with the use of the new highly processive and thermostable Invitrogen™ SuperScript™ IV Reverse Transcriptase (RT) enzyme, which allows cDNA reaction to occur at higher temperatures and in less time. The SuperScript IV RT gives the highest cDNA yields and sensitivity even with suboptimal purity or scarce templates. With the SuperScript IV VILO Master Mix, the RT-qPCR workflow is further accelerated with the extremely fast and simple gDNA removal approach. It dramatically reduces the reverse transcription protocol time and reduces variation related to possible RNA loss or damage during the conventional DNase step. SuperScript IV VILO Master Mix is your new tool to enable more efficient and reproducible RT-qPCR.

Sensitivity and perfect linearity in a 10-minute reaction

The high processivity of SuperScript IV RT enzyme in SuperScript IV VILO Master Mix allows completion of RT reaction in 10 minutes. In this short reaction time, the master mix is capable of generating higher cDNA yields compared to those obtained by lengthier competitor protocols (Figure 1)—keeping the perfect linearity typical for VILO™ products (Figure 2). We compared several different cDNA synthesis kits in fourteen different RT-qPCR assays using low starting-RNA input and found SuperScript IV VILO Master Mix produced highest efficiency results. Compared with other competitors, SuperScript IV VILO Master Mix consistently lowered C_t values by >2 cycles (Figure 4).

Facts

- Super fast—RT reaction in 10 minutes and gDNA removal in 2 minutes
- Super high yield—over 2 cycles of lowered C_t values ahead of all other reverse transcription reagents
- Super convenient one-tube reaction master mix for 2-step RT-qPCR
- Super sensitive even with low template amounts and suboptimal purity samples

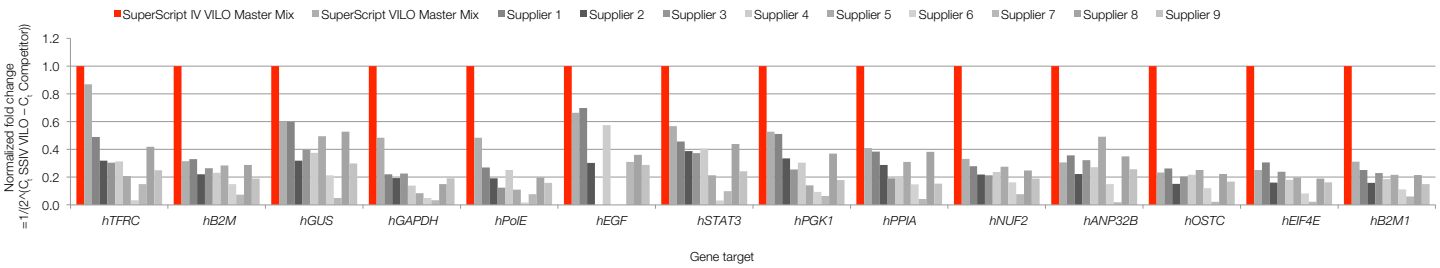

Figure 1. Highest sensitivity with SuperScript IV VILO Master Mix across 14 TaqMan gene targets. RT efficiency of SuperScript IV VILO Master Mix compared with ten commercial first strand cDNA synthesis master mixes by RT-qPCR. Master mixes were used to synthesize cDNA in 20 µL RT reactions, per manufacturer instructions, using 1 ng of total HeLa RNA input. For qPCR, 1 µL of RT reactions was used in 10 µL Invitrogen™ EXPRESS qPCR SuperMix (Cat. No. 11785200) reactions with Applied Biosystems™ TaqMan™ primer and/or probes for gene targets indicated. qPCR results are shown normalized to fold change relative to SuperScript IV VILO Master Mix [=1/(2^(C_t SSIV VILO – C_t Competitor))].

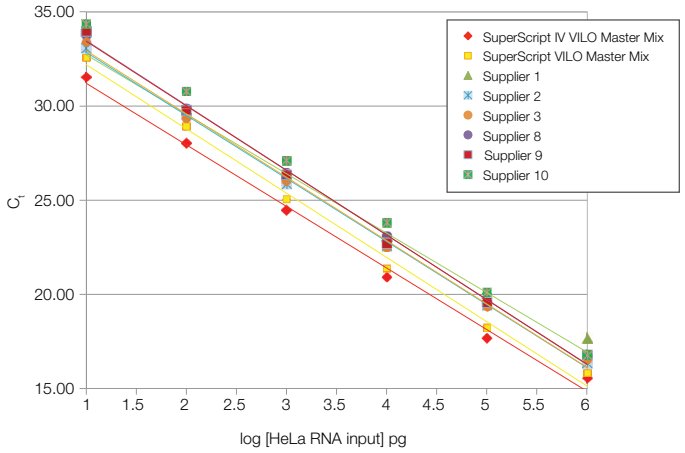

Figure 2. Perfect linearity and lower C_t value with SuperScript IV VILO Master Mix. SuperScript IV VILO Master Mix compared by RT-qPCR with seven commercial master mixes using 5 orders of magnitude (10 pg–1 µg) of total HeLa RNA input in 20 µL RT reactions. Linearity for Applied Biosystems™ TaqMan™ GAPDH target shown in graph corresponds to slope and R^2 calculated from C_t values for each formulation as determined by qPCR in 10 µL reactions using EXPRESS qPCR SuperMix (SuperScript IV VILO Master Mix efficiency = 102.1%, slope = -3.3, and R^2 = 0.99).

Improved performance on challenging samples

With high processivity and affinity to RNA, SuperScript IV RT has been shown to result in improved cDNA synthesis performance on samples that usually are considered challenging, such as degraded RNA and/or RNA containing inhibitors.

To demonstrate the efficiency of SuperScript IV VILO Master Mix in the presence of a variety of inhibitors, SuperScript IV VILO Master Mix was compared with other commercially available cDNA synthesis kits in RT-qPCR. C_t values generated by competing kits were normalized to C_t values of SuperScript IV VILO Master Mix using the equation: Normalized Y values = [1/(2^(C_t SSIV VILO – C_t Competitor))]. As exhibited in Figure 3A, SuperScript IV VILO had the highest efficiency among all cDNA synthesis kits in the presence or absence of inhibitors shown.

Similarly, when SuperScript IV VILO Master Mix is tested with RNA purified from frozen tissue samples that commonly cause low reverse transcription efficiency, improved performance over other cDNA synthesis kits or master mixes is also observed (Figure 3B).

Consistently higher cDNA yields across a variety of gene targets

To evaluate the efficiency of SuperScript IV VILO Master Mix on a wide variety of gene targets, we compared it to other cDNA synthesis kits for RT-qPCR applications using a panel of 96 gene targets (Figure 4). ΔC_t values for SuperScript IV VILO Master Mix and competitors are depicted in the graph. For 96% of targets evaluated, SuperScript IV VILO Master Mix achieved greater than 2 C_t values lower than competitors and SuperScript VILO Master Mix, and overall generates higher cDNA yields.

Learn more at **thermofisher.com/4vilo**

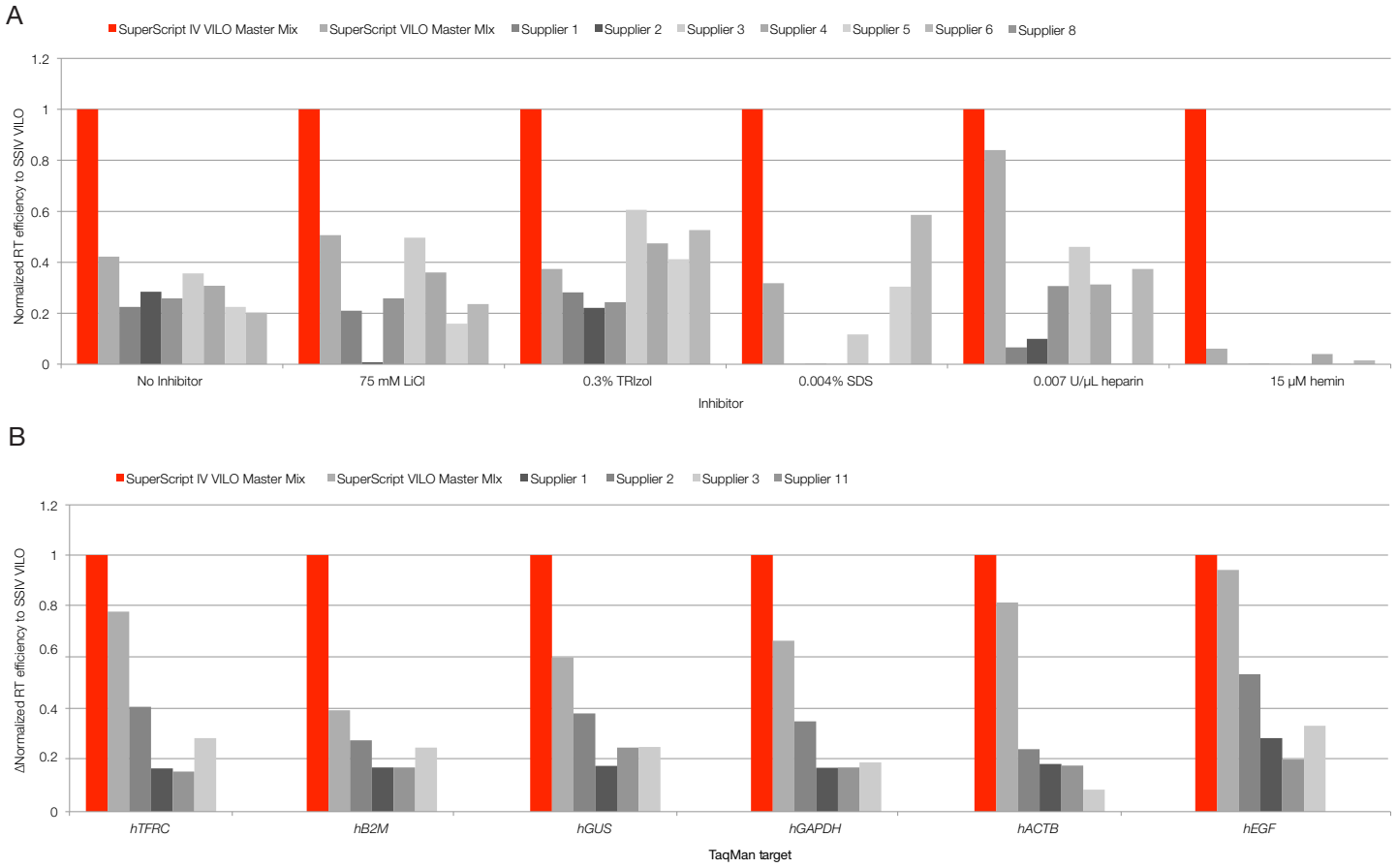

Figure 3. Higher RT efficiency on challenging samples using SuperScript IV VILO Master Mix. (A) SuperScript IV VILO Master Mix was compared to eight other master mixes.using 100 ng total HeLaRNA in 20 µL RT reactions plus or minus the concentration of the inhibitors indicated. Performance in qPCR was evaluated in 10 µL reactions with TaqMan primer and/or probes for the *B2M* gene targets using EXPRESS qPCR SuperMix. **(B)** SuperScript IV VILO Master Mix was compared with five other commercial master mixes using 50 ng of RNA derived from frozen tissue RNA in 20 µL RT reactions. Performance in qPCR was evaluated in 10 µL reactions with TaqMan primer and/or probes for the six gene targets using EXPRESS qPCR SuperMix.

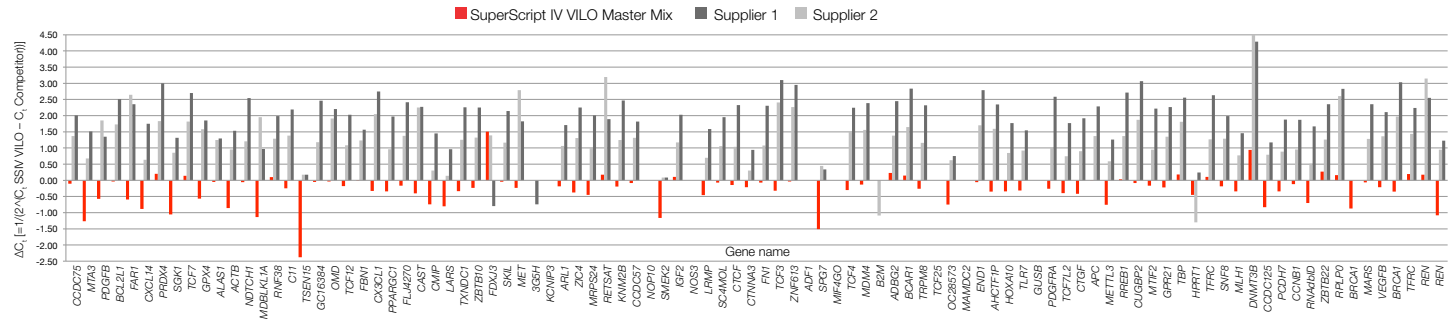

Figure 4. Higher cDNA yield with SuperScript IV VILO Master Mix in Applied Biosystems™ TaqMan™ RT-qPCR gene panel analysis. Comparison of SuperScript IV VILO Master Mix with two commercial master mixes in an TaqMan RT-qPCR gene panel analysis using 100 ng total HeLa RNA in 20 µL RT reaction mixture. Performance in qPCR was evaluated in 10 µL reactions with TaqMan primer and/or probes for the gene targets indicated using EXPRESS qPCR SuperMix.

Integrated gDNA removal step for accurate quantification

Many RNA isolation kits and methods do not generate RNA that is completely DNA-free; therefore gDNA elimination remains a critical step in RT-qPCR workflow as residual DNA may lead to false-positive, higher background or lower assay sensitivity. The SuperScript IV VILO Master Mix with Invitrogen™ ezDNase provides a simplified workflow that includes a 2-minute genomic DNA elimination step and excludes a DNase thermal inactivation or removal step (Figure 5). ezDNase, a novel double-strand-specific, thermolabile DNase, is engineered to remove contaminating genomic DNA without affecting quality or quantity of target RNA or damaging single-stranded DNA such as primers and probes.

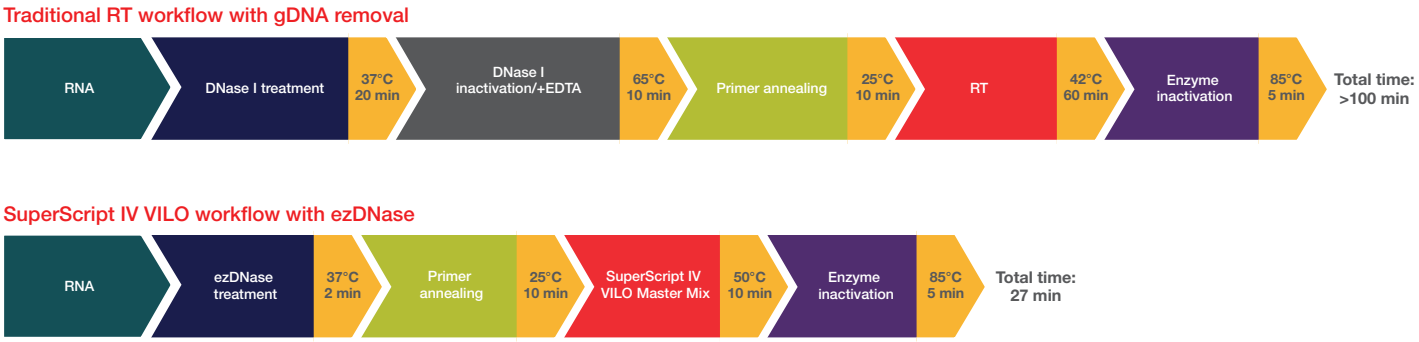

Figure 5. A timeline comparison between the use of traditional DNase I (top) and ezDNase (bottom).

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Ordering information

Product	Size	Cat. No.
SuperScript IV VILO Master Mix: Includes 5x master mix containing SuperScript IV RT, RNase inhibitor, proprietary helper protein, random primers, oligo(dT), MgCl2 and dNTPs; no-RT control master mix; DEPC-treated water	50 reactions 500 reactions	11756050 11756500
SuperScript IV VILO Master Mix with ezDNase: Includes 5x master mix containing SuperScript IV RT, RNase inhibitor, proprietary helper protein, random primers, oligo(dT), MgCl2 and dNTPs; no-RT control master mix; ezDNase; ezDNase buffer; DEPC-treated water	50 reactions 500 reactions	11766050 11766500
SuperScript IV First-Strand Synthesis System: Includes Superscript IV RT; 5x RT buffer; RNase inhibitor; Random hexamers; Oligo(dT); dNTPs; RNase H, HeLa RNA; Sense control primer; Antisense control primer; DEPC-treated water	50 reactions 200 reactions	18091050 18091200
SuperScript IV Reverse Transcriptase: Supplied with 5x RT buffer	2,000 units 10,000 units 4 x 10,000 units	18090010 18090050 18090200
ezDNase: Supplied with 10x ezDNase buffer	50 reactions	11766051

Every step of your experiment is important. We are here to help you with complete solutions for every step of your molecular biology workflow, starting from RNA isolation, into reverse transcription, PCR, thermal cycling, electrophoresis, and quantitation. Discover our innovative and high-quality products to help streamline your experiments. Learn more or get a copy of the molecular biology handbook at **thermofisher.com/everystepcounts**

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