

 Contents

Reagents provided are sufficient for 50, 500, and 2000 20-μL RT reactions.

Component	SuperScript™ V VILO™ Master Mix, Primer-Free			SuperScript™ V VILO™ Master Mix, Primer-Free, with gDNA removal		
	Cat. No. 11900001	Cat. No. 11900002	Cat. No. 11900003	Cat. No. 11900111	Cat. No. 11900112	Cat. No. 11900113
5X SuperScript™ V VILO™ Master Mix, Primer-Free [1]	200 μL	2 × 1 mL	8 × 1 mL	200 μL	2 × 1 mL	8 × 1 mL
5X SuperScript™ V VILO™ Master Mix, No RT Control, Primer-Free	100 μL	1 mL	4 × 1 mL	100 μL	1 mL	4 × 1 mL
Nuclease-free water	1.25 mL	1.25 mL	4 × 1.25 mL	1.25 mL	1.25 mL	4 × 1.25 mL
10X ezDNase™ Master Mix	—	—	—	50 μL	500 μL	4 × 500 μL

[1] Additional master mix sufficient for 1250 reactions (5 mL) can be purchased separately (Cat. No. 11900004).

 Storage

The product may be shipped at ambient temperature, with cool packs, or on dry ice.

Store all contents at:

- -20°C for long-term storage until expiry date
- +4°C for up to 5 months
- Room temperature (+20±5°C) for up to 1 month

 Product description

The Invitrogen™ SuperScript™ V VILO™ Master Mix, Primer-Free is an optimized solution for first strand cDNA synthesis.

- The 5X master mix includes SuperScript™ V Reverse Transcriptase, RNaseOUT™ Recombinant RNase Inhibitor, helper proteins, stabilizer proteins, MgCl₂, and dNTPs.
- A No RT Control contains all the components of the master mix except the reverse transcriptase enzyme. The No RT Control is used to determine the level of genomic DNA (gDNA) contamination in the RNA sample.
- 10X ezDNase™ Master Mix is provided as a convenient formulation to remove gDNA contamination from template RNA prior to the RT reaction.

 Online resources

- Visit [thermofisher.com](https://www.thermofisher.com) for additional information and protocols.
- For support, visit [thermofisher.com/support](https://www.thermofisher.com/support).

For Research Use Only. Not for use in diagnostic procedures.

 General guidelines for handling RNA

- Use proper microbiological aseptic technique.
- Wear gloves while handling reagents, materials and RNA samples to prevent RNase contamination.
- Use disposable individually wrapped, sterile plasticware for all procedures.
- Use aerosol-resistant pipette tips.
- Dedicate a separate set of pipettes, buffers and enzymes for RNA work.
- Use RNase-free microcentrifuge tubes.

 Guidelines for RNA preparation

RNA isolation

Isolate total RNA using TRIzol™ Reagent, the PureLink™ RNA Mini Kit, or the MagMAX™-96 Total RNA Isolation Kit.

RNA quality

Use high-quality, intact RNA for accurate quantification. RNA should be free of RNase contamination and handled using aseptic techniques to prevent degradation.

RNA quality assessment

Assess RNA integrity using a bioanalyzer or by agarose gel electrophoresis.



Required materials

- Template: RNA
- Primers: Oligo(dT)20 Primer (Cat. No. 18418020), Random Hexamers (Cat. No. N8080127), or gene-specific primers

Guidelines for primer selection

Oligo(dT) primers

Short stretches of 12–20 thymidines that anneal to poly(A) tails of eukaryotic mRNA. They are ideal for full-length cDNA synthesis, cDNA library preparation, and 3' RACE applications. However, they are not suitable for degraded RNA, non-polyadenylated RNA (e.g., prokaryotic or miRNA), or applications where minimizing 3' bias is important. Anchored oligo(dT) variants (such as Anchored Oligo(dT)₂₀ Primer, Cat. No. 12577011) help reduce internal priming and slippage, improving specificity at the start of the poly(A) tail.

Random primers

- Short, random oligonucleotides—most commonly hexamers, though longer versions such as dodecamers can be used—represent the least specific priming strategy. They are typically chosen when full-length mRNA is difficult to copy. In this approach, all RNA species in the sample can serve as templates for first-strand cDNA synthesis, with target specificity introduced later by gene-specific PCR primers.
- Random primers are well suited for degraded RNA, non-polyadenylated RNA, and highly structured RNA (e.g., viral genomes).
- To optimize cDNA length and yield, the ratio of random primers to RNA should be determined empirically for each preparation.
- As a general guideline, 2 μ M of random hexamers is sufficient for most applications involving total RNA. Increasing the concentration to 5 μ M may enhance the yield of short cDNA products (<500 bp) but can reduce the yield of longer and full-length transcripts.

Combination of oligo(dT) and random hexamers

Commonly used in two-step RT-PCR and RT-qPCR to achieve both broad transcript coverage and mRNA specificity. Random primers enable cDNA synthesis from a wide range of RNA species, including partially degraded transcripts, while oligo(dT) primers ensure efficient targeting of polyadenylated mRNA. This combined approach improves overall cDNA yield and representation.

Gene-specific primers

Designed to anneal to a defined target sequence, providing the highest level of specificity in cDNA synthesis. This approach requires a unique primer for each gene of interest and typically more input RNA per target, as separate reverse transcription reactions are needed for each assay.

	Optimal concentration	Recommended concentration range ^[1]	Key benefits
Oligo(dT)	2.5 μ M	2–5 μ M	Full-length reverse transcription of RNA with poly(A) tail
Random hexamers	2.5 μ M	2–5 μ M	Reverse transcription of most RNA species, including degraded RNA
Oligo(dT) and random hexamers	1.25 μ M oligo(dT) and 0.75 μ M random hexamers	0.5–2.5 μ M each (up to 5 μ M)	Combined benefits of oligo(dT) and random primers
Gene-specific primer	0.1 μ M	0.1–1.0 μ M	Reverse transcription specific to the gene of interest

[1] For specific applications, primer concentration can be optimized within the suggested range.

Guidelines for reverse transcription

Genomic DNA control

To determine the level of genomic DNA (gDNA) contamination, include a No-RT control by setting up a reaction using the SuperScript™ V VILO™ Master Mix, No RT Control, Primer Free.

RNA input

Use from 100 fg to 5 µg of total RNA, or up to 500 ng mRNA as starting material in a 20 µL reaction.

High RNA input

SuperScript™ V VILO™ Master Mix, Primer Free efficiently synthesizes cDNA from high RNA inputs (up to 5 µg total RNA in a 20 µL reaction). However, such high-input cDNA is often not suitable for downstream applications such as end-point PCR or qPCR, which typically require lower template amounts. Therefore, dilute the cDNA after reverse transcription according to the recommendations of the downstream application protocol.

Adjusting reaction volume

For high RNA inputs (>1 µg), the RT reaction volume can be increased, if necessary, up to 100 µL.

Reaction setup conditions

Reverse transcription reactions can be assembled at room temperature without negatively affecting RT performance. For precious or lower-quality RNA samples, it is recommended to keep the RNA on a cold rack prior to RT setup to minimize degradation.

RNA template removal

Amplification of some PCR targets (>1 kb) may require removal of RNA. To remove RNA add 1 µL of Ribonuclease H (Cat. No. 18021014) after cDNA synthesis and incubate at 37°C for 20 minutes.

Protocol optimization

Test different temperature and time conditions to optimize the protocol for your application.

SuperScript™ V Reverse Transcriptase is highly active across a broad temperature range of 37–65°C. Lower temperatures may improve primer annealing, while higher temperatures may benefit GC-rich or structured RNA and improve gene-specific priming.

- Vary incubation temperature from 37–65°C.

Longer incubation time may help with low-input, low-abundance, long, or difficult templates. Short incubations may suit rapid workflows with abundant short targets but should be validated against the standard protocol.

- Vary incubation time from 1–60 minutes.

Thermal cycler settings

When performing RT in a thermal cycler, set the heated lid temperature to 90–95°C.

Automation Workflows

For automated workflows, assembled reactions can be stored at room temperature prior to the reverse transcription step for:

- Up to 24 hours when using a probe-based qPCR system.
- Up to 4 hours when using a SYBR™ Green-based qPCR system.

Guidelines for long cDNA synthesis (>15 kb) and GC-rich RNA

(Optional) Primer annealing

Perform a primer annealing at 65°C prior to reverse transcription to improve full length cDNA synthesis. Mix the RNA template with primers and water up to 16 µL and incubate at 65°C for 5 minutes. Immediately chill on ice, briefly centrifuge, add SuperScript™ V VIL0™ Master Mix, Primer-Free. Mix, briefly centrifuge and proceed to "Reverse transcribe RNA" step 4" on [page 5](#) or "step 6" on [page 6](#) in the reverse transcription protocols. This step is also beneficial for templates with high GC content or templates known to contain secondary structures.

Reverse transcription time

Extend the reverse transcription step for up to 60 minutes to improve the yield of long cDNA products.

RNA quality

Use high-quality, freshly prepared RNA for reverse transcription. Minimize freeze–thaw cycles, as RNA degradation can significantly reduce long cDNA yield.

Guidelines for performing template switching

RNA input

The recommended RNA input range is 10 pg to 1 µg. The most efficient template switching is typically achieved with 1 ng to 1 µg of input RNA.

Reverse transcription primer options and concentrations

Oligo(dT) primers, gene-specific primers (GSP), or other custom-designed RT primers can be used. Recommended final primer concentrations in the RT reaction are ≥4 µM for the RT primer and ≥5.5 µM for the template-switching oligonucleotide (TSO).

Reverse transcription times

Extend the RT step to 30–60 minutes at 50 °C to improve template-switching efficiency.

Guidelines for qPCR amplification

Recommended qPCR master mix

Prepared cDNA is compatible with most commercially available master mixes. However, for optimal performance the following reagents are recommended.

- **TaqMan system**

TaqMan™ Fast Advanced Master Mix for qPCR (Cat. No. 4444556).

- **SYBR Green system**

PowerTrack™ SYBR™ Green Master Mix for qPCR (Cat. No. A46012).

Template input

Start with sufficient template to ensure detectable amplification within 35 cycles (typically >10⁴ copies, but <5 µg of total RNA used for cDNA synthesis).

gDNA contamination control

Include a No-RT control to verify the absence of genomic DNA (gDNA) contamination. Use No-RT cDNA equivalent to ~10% of the qPCR reaction volume.

High GC-content templates

For templates with high GC content, perform an initial 97°C dissociation step for several cycles to generate single-stranded DNA prior to PCR amplification.

qPCR with fluorescent probes or primers

When using fluorescent primers or probe-based assays, up to 10% of the qPCR reaction volume can consist of undiluted cDNA from the RT reaction mix.

qPCR with SYBR™ Green reagents

- If starting with 100 ng of total RNA, undiluted cDNA from the RT reaction can constitute up to 10% of the total qPCR reaction volume.
- If starting with >100 ng total RNA, test a serial dilution of the cDNA to determine optimal performance, as high cDNA concentrations may affect the SYBR™ Green baseline signal.

Perform RT protocol using SuperScript™ V VILO™ Master Mix, Primer-Free

The following example shows appropriate volumes for a single 20-μL reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, then dispense appropriate volumes into each reaction tube (See [page 6](#) for a RT protocol including gDNA removal).

Steps	Action	Procedure details																		
1 	Thaw reagents	If necessary, thaw, mix, and briefly centrifuge each component before use.																		
2 	Prepare RT and No RT Control reaction mix	Add the following components to a microcentrifuge tube. <table border="1"> <thead> <tr> <th>Component</th><th>RT rxn</th><th>No RT control</th></tr> </thead> <tbody> <tr> <td>5X SuperScript™ V VILO™ Master Mix, Primer-Free</td><td>4 μL</td><td>—</td></tr> <tr> <td>5X SuperScript™ V VILO™ Master Mix, No RT Control, Primer-Free</td><td>—</td><td>4 μL</td></tr> <tr> <td>50 μM Oligo(dT)20 primer, OR 50 μM random hexamers, OR 2 μM gene-specific primer</td><td>1 μL</td><td>1 μL</td></tr> <tr> <td>Template RNA</td><td colspan="2">100 fg–5 μg total RNA up to 500 ng mRNA</td></tr> <tr> <td>Nuclease-free water</td><td>to 20 μL</td><td>to 20 μL</td></tr> </tbody> </table> Mix gently, then briefly centrifuge the reaction mix.	Component	RT rxn	No RT control	5X SuperScript™ V VILO™ Master Mix, Primer-Free	4 μL	—	5X SuperScript™ V VILO™ Master Mix, No RT Control, Primer-Free	—	4 μL	50 μM Oligo(dT)20 primer, OR 50 μM random hexamers, OR 2 μM gene-specific primer	1 μL	1 μL	Template RNA	100 fg–5 μg total RNA up to 500 ng mRNA		Nuclease-free water	to 20 μL	to 20 μL
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3  25°C  2 min	(Optional) Anneal primers	Incubate at 25°C for 0–2 minutes if using random hexamer primers. Note: Skip this step if using other primer types or if reactions are set up at room temperature.																		
4  50°C  10 min	Reverse transcribe RNA	Incubate at 50°C for 10 minutes. Note: 10 minutes at 50°C is optimal for most targets. However, both reaction time and temperature can be optimized (see "Protocol optimization" on page 3).																		
5  95°C  1 min	Inactivate enzyme	- Incubate at 95°C for 1 minute if cDNA is used for PCR or qPCR. - Incubate at 85°C for 5 minutes if cDNA is used for other applications or visualized on gel electrophoresis.																		
6  	qPCR/PCR amplification	Use the diluted or undiluted cDNA immediately for qPCR or PCR amplification or store at –20°C for up to one week, or –70°C for long-term storage.																		

Perform RT protocol using SuperScript™ V VILO™ Master Mix, Primer-Free, with gDNA removal

The following example shows appropriate volumes for a single 20-µL reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, then dispense appropriate volumes into each reaction tube (See [page 5](#) for a RT protocol without gDNA removal).

Steps	Action	Procedure details															
1 	Thaw reagents	If necessary, thaw, mix, and briefly centrifuge each component before use.															
2 	Prepare gDNA digestion reaction mix	Add the following components to a microcentrifuge tube for each RT or No RT Control reaction. <table><tr><th>Component</th><th>ezDNase™ rxn</th></tr><tr><td>10X ezDNase™ Master Mix</td><td>1 µL</td></tr><tr><td>Template RNA</td><td>100 fg–5 µg total RNA or up to 500 ng mRNA</td></tr><tr><td>Nuclease-free water</td><td>to 10 µL</td></tr></table>	Component	ezDNase™ rxn	10X ezDNase™ Master Mix	1 µL	Template RNA	100 fg–5 µg total RNA or up to 500 ng mRNA	Nuclease-free water	to 10 µL							
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3  37°C  2 min	Digest gDNA	▪ Mix gently, then centrifuge briefly. Incubate at 37°C for 2 minutes. Centrifuge and place the tubes on ice. ▪ <i>(Optional)</i> For RT-PCR of targets >3 kb incubate at 55°C for 5 minutes to inactivate the enzyme prior to RT.															
4 	Prepare RT and No RT Control reaction mix	Add the following components to the microcentrifuge tube containing the gDNA treated RNA. <table><tr><th>Component</th><th>RT rxn</th><th>No RT control</th></tr><tr><td>5X SuperScript™ V VILO™ Master Mix, Primer-Free</td><td>4 µL</td><td>—</td></tr><tr><td>5X SuperScript™ V VILO™ Master Mix, No RT Control, Primer-Free</td><td>—</td><td>4 µL</td></tr><tr><td>50 µM Oligo(dT)20 primer, OR 50 µM random hexamers, OR 2 µM gene-specific primer</td><td>1 µL</td><td>1 µL</td></tr><tr><td>Nuclease-free water</td><td>5 µL</td><td>5 µL</td></tr></table> Mix gently, then briefly centrifuge the reaction mix.	Component	RT rxn	No RT control	5X SuperScript™ V VILO™ Master Mix, Primer-Free	4 µL	—	5X SuperScript™ V VILO™ Master Mix, No RT Control, Primer-Free	—	4 µL	50 µM Oligo(dT)20 primer, OR 50 µM random hexamers, OR 2 µM gene-specific primer	1 µL	1 µL	Nuclease-free water	5 µL	5 µL
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5  25°C  2 min	<i>(Optional)</i> Anneal primers	Incubate at 25°C for 0–2 minutes if using random hexamer primers. Note: Skip this step if using other primer types or if reactions are set up at room temperature.															
6  50°C  10 min	Reverse transcribe RNA	Incubate at 50°C for 10 minutes. Note: 10 minutes at 50°C is optimal for most targets. However, both reaction time and temperature can be optimized (see "Protocol optimization" on page 3).															
7  95°C  1 min	Inactivate enzyme	▪ Incubate at 95°C for 1 minute if cDNA is used for PCR or qPCR. ▪ Incubate at 85°C for 5 minutes if cDNA is used for other applications or visualized on gel electrophoresis.															
8 	qPCR/PCR amplification	Use the diluted or undiluted cDNA immediately for qPCR or PCR amplification or store at –20°C for up to one week, or –70°C for long-term storage.															

Troubleshooting

Observation	Cause	Recommended action
No product after reverse transcription	Problem with first-strand cDNA synthesis	Use high quality RNA, such as Cervical Adenocarcinoma (HeLa-S3) Total RNA (Cat. No. AM7852), as a control to confirm efficiency of first-strand reaction.
	Reverse transcription inhibitors present in RNA	In samples containing inhibitors, reducing the amount of input RNA and/or cDNA may improve cDNA synthesis and/or PCR performance. ▪ Perform serial dilution of RNA input in the reverse transcription reaction. ▪ Increase reverse transcription time up to 60 minutes. ▪ Perform serial dilution of the cDNA prior to adding it to the PCR mix.
	Low copy or degraded RNA sample	Increase reverse transcription time up to 60 minutes.
	Target RNA contains strong secondary structure	▪ Increase RT reaction temperature stepwise from 55–65°C to determine optimal conditions (the reverse transcriptase has activity up to 65°C).
Unexpected product after cDNA synthesis	Genomic DNA contamination of RNA sample	▪ Perform a No-RT control reaction using the SuperScript™ V VILO™ Master Mix, No RT Control, Primer-Free to assess the level of genomic DNA contamination in the RNA sample. ▪ Pretreat the RNA sample with 10X ezDNase™ Master Mix if included in kit. Otherwise use DNase I, Amplification Grade (Cat. No. 18068015) or ezDNase™ Enzyme (Cat. No. 11766051). ▪ Design PCR primers that anneal to exons flanking an intron or span an exon–exon junction to distinguish amplified cDNA from contaminating genomic DNA.
	Non-specific annealing of PCR primers	▪ Use a hot-start PCR enzyme. ▪ Optimize the PCR annealing temperature to improve specificity. ▪ Optimize the magnesium concentration.
Cloudiness and/or white precipitate in RT reaction	Evaporation of RT reaction	▪ Cloudiness or formation of a white precipitate that may result from evaporation during the reverse transcription reaction does not affect RT performance. ▪ Ensure that tubes or plates are sealed tightly to minimize evaporation during incubation. ▪ Optimal RT results are obtained when evaporation and condensation is minimized. This can be achieved by using shorter incubation times when possible or performing the reaction in a thermal cycler with a heated lid.

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