RevertAid RT Kit

Catalog Number K1691

Pub. No. MAN0012955 **Rev.** B00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

Contents

Cat. No.	Contents	Amount	Storage
K1691 (500 rxns)	RevertAid RT (200 U/µL)	5 × 120 µL	-25 °C to -15 °C
	RiboLock RNase Inhibitor (20 U/µL)	5 × 120 µL	
	5X Reaction Buffer 250 mM Tris-HCl (pH 8.3), 250 mM KCl, 20 mM $MgCl_2$, 50 mM DTT	5 × 500 µL	
	10 mM dNTP Mix	4 × 250 µL	
	Random Hexamer Primer, 100 µM	5 × 120 µL	
	Water, nuclease-free	6 × 1.25 mL	

Description

The Thermo Scientific™ RevertAid™ RT Kit is a complete system for efficient synthesis of first strand cDNA from mRNA or total RNA templates. The kit uses RevertAid Reverse Transcriptase (RT), which has lower RNase H activity compared to AMV reverse transcriptase. The enzyme maintains activity at 42-50 °C and is suitable for synthesis of cDNA up to 13 kb.
The recombinant Thermo Scientific™ RiboLock™ RNase Inhibitor, supplied with the kit, effectively protects RNA from degradation at temperatures up to 55 °C.
First strand cDNA synthesized with this system can be directly used as a template in PCR or real-time PCR. It is also ideal for second strand cDNA synthesis or linear RNA amplification. Radioactively and non-radioactively labeled nucleotides can be incorporated into first strand cDNA for use as a probe in hybridization experiments, including microarrays.

Important Notes

Avoiding ribonuclease contamination

RNA purity and integrity are essential for synthesis of full-length cDNA. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment.
General recommendations to avoid RNase contamination:

- DEPC-treat all tubes and pipette tips to be used in cDNA synthesis or use certified nuclease-free labware.
- Wear gloves when handling RNA and all reagents, as skin is a common source of RNases. Change gloves frequently.
- Use RNase-free reagents, including high quality water (e.g., Water, nuclease-free, #R0581).
- Use RiboLock RNase Inhibitor (provided with the kit) to protect RNA from the activity of RNases.
- Keep all kit components tightly sealed when not in use. Keep all tubes tightly closed during the reverse transcription reaction.

Template RNA

Total cellular RNA isolated by standard methods is suitable for use with the kit. Purified RNA must be free of salts, metal ions, ethanol and phenol to avoid inhibiting the cDNA synthesis reaction. Trace contaminants can be removed by ethanol precipitation of the RNA followed by two washes of the pellet with cold 75 % ethanol.
For RT-PCR applications, template RNA must be free of DNA contamination. Prior to cDNA synthesis, RNA can be treated with DNase I, RNase-free (#EN0521) to remove trace amounts of DNA. Always perform a control (RT-minus) reaction which includes all components for RT-PCR except for the reverse transcriptase enzyme.

Removal of genomic DNA from RNA preparations

1. Add to an RNase-free tube:

Component	Volume
RNA	1 µg
10X Reaction Buffer with $MgCl_2$	1 µL
DNase I, RNase-free (#EN0521) *	1 µL (1 U)
Water, nuclease-free	to 10 µL

* Do not use more than 1 U of DNase I, RNase-free per 1 µg of RNA.

2. Incubate at 37 °C for 30 min.
3. Add 1 µL 50 mM EDTA and incubate at 65 °C for 10 min. RNA hydrolyzes during heating with divalent cations in the absence of a chelating agent (1). Alternatively, use phenol/chloroform extraction.
4. Use the prepared RNA as a template for reverse transcriptase.

RNA sample quality

Assess RNA integrity prior to cDNA synthesis. The most common method is denaturing agarose gel electrophoresis followed by ethidium bromide staining. If both 18S and 28S rRNA appear as sharp bands after electrophoresis of total eukaryotic RNA, the RNA is considered to be intact. The 28S rRNA band should be approximately twice as intense as the 18S rRNA. Any smearing of rRNA bands is an indication of degraded mRNA. If this occurs, a new sample of total RNA should be prepared.

RNA quantity

- Use 0.1 ng - 5 µg of total RNA or 10 pg - 500 ng of poly(A) mRNA to generate first strand cDNA as the initial step of a two-step RT-PCR protocol.
- Use 1 µg of isolated mRNA to generate first strand cDNA for second-strand synthesis and subsequent cloning reactions.

Protocols

Please read the Important Notes before starting.

I. First Strand cDNA Synthesis

After thawing, mix and briefly centrifuge the components of the kit. Store on ice.

1. Add the following reagents into a sterile, nuclease-free tube on ice in the indicated order:

Component	Volume
Template RNA total RNA *or* poly(A) mRNA *or* specific RNA	 0.1 ng - 5 µg 10 pg - 0.5 µg 0.01 pg - 0.5 µg
Primer* Random Hexamer primer	1 µL
Water, nuclease-free	to 12 µL
Total volume	**12 µL**

*1 µL of 100 µM Oligo$(dT)_{18}$ primer (#SO131) or gene-specific primer (15-20 pmol) can also be used.

2. *Optional.* If the RNA template is GC-rich or contains secondary structures, mix gently, centrifuge briefly and incubate at 65 °C for 5 min. Chill on ice, spin down and place the vial back on ice.
3. Add the following components in the indicated order:

Component	Volume
5X Reaction Buffer	4 µL
RiboLock RNase Inhibitor (20 U/µL)	1 µL
10 mM dNTP Mix	2 µL
RevertAid RT (200 U/µL)	1 µL
Total volume	**20 µL**

4. Mix gently and centrifuge briefly.
5. For oligo(dT)$_{18}$ or gene-specific primed cDNA synthesis, incubate for 60 min at 42 °C.

For random hexamer primed synthesis, incubate for 5 min at 25 °C followed by 60 min at 42 °C.

Note. For GC-rich RNA templates the reaction temperature can be increased up to 45 °C.

6. Terminate the reaction by heating at 70 °C for 5 min.

The reverse transcription reaction product can be directly used in PCR applications or stored at -20 °C for less than one week. For longer storage, -70 °C is recommended

II. PCR Amplification of First Strand cDNA

The product of the first strand cDNA synthesis can be used directly in PCR or qPCR. The volume of first strand cDNA synthesis reaction mixture should not comprise more than 1/10 of the total PCR reaction volume. Normally, 2 µL of the first strand cDNA synthesis reaction mixture is used as template for subsequent PCR in 50 µL total volume.

Control Reactions

Positive and negative control reactions should be used to verify the results of the first strand cDNA synthesis steps.

- **Reverse transcriptase minus (RT-) negative control** is important in RT-PCR or RT-qPCR reactions to assess for genomic DNA contamination of the RNA sample. The control RT- reaction contains every reagent for the reverse transcription reaction except for the RT enzyme.
- **No template negative control (NTC)** is important to assess for reagent contamination. The NTC reaction contains every reagent for the reverse transcription reaction except for RNA template.

Troubleshooting

Low yield or no RT-PCR product
Degraded RNA template. RNA purity and integrity is essential for synthesis of full-length cDNA. Always assess the integrity of RNA prior to cDNA synthesis. Sharp 18S and 28S RNA bands should be visible after denaturing agarose gel electrophoresis of total eukaryotic RNA. Follow general recommendations to avoid RNase contamination. **Low template purity.** Trace amounts of agents used in RNA purification protocols may remain in solution and inhibit first strand synthesis, e.g., SDS, EDTA, guanidine salts, phosphate, pyrophosphate, polyamines, spermidine. To remove trace contaminants, re-precipitate the RNA with ethanol and wash the pellet with 75 % ethanol. **Insufficient template quantity.** Increase the amount of template to the recommended level. Following DNase I treatment, terminate the reaction by heat inactivation in the presence of EDTA (to bind magnesium ions), RNA hydrolyzes during heating in the absence of a chelating agent (1). **Incorrect primer choice.** Use the correct primer for the RNA template. Use the random hexamer primer instead of the oligo(dT)$_{18}$ primer with bacterial RNA or RNA without a poly(A) tail. Ensure sequence-specific primers are complementary to 3'-end of the template RNA. **GC rich template.** If the RNA template is GC rich or is known to contain secondary structures, increase the temperature of the reverse transcription reaction up to 45 °C.
RT-PCR product longer than expected
RNA template is contaminated with DNA. Amplification of genomic DNA containing introns. Perform DNase I digestion prior reverse transcription. To avoid amplification of genomic DNA, design PCR primers on exon-intron boundaries.
RT-PCR product in negative control
RNA template is contaminated with DNA. PCR product in the negative control (RT-) indicates the reaction is contaminated with DNA. Perform DNase I digestion prior reverse transcription.

Reference

1. Wiame, I., et al., Irreversible heat inactivation of DNaseI without RNA degradation, BioTechniques, 29, 252-256, 2000.

Revision history: Pub. No. MAN0012955

Revision	Date	Description
B00	2024-04-08	Revized user guide template and removed COA content

Limited product warranty

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