



AccelerRT® III Reverse Transcriptase User Manual

AccelerRT® III Reverse Transcriptase

Cat. No: PC026 (10,000U) , PC027 (50,000U)

User Manual

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AccelerRT® III Reverse Transcriptase

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I. Description

AccelerRT® III Reverse Transcriptase(RT) is an engineered version of M-MuLV RT with reduced RNase H activity. RNase H specifically degrades the RNA strand in RNA-DNA hybrids, therefore it probably degrades the RNA template in RNA-DNA hybrids when the first-strand cDNA synthesis. The enzyme can be used to synthesize more full-length product than conventional M-MLV, stronger extension ability. And it can be used to Real Time RT-PCR.

■ Source

The gene encoding a mutant M-MLV Reverse Transcriptase(RNase H-) is expressed in E.coli.

■ Definition of Activity Unit

One unit incorporates 1 nmole of dTTP into acid-precipitable material in 10min at 37°C using poly — poly(A)oligo(dT) as template-primer.

■ Quality Guarantee

No RNase activity was detected.

■ Applications

- ✧ Reverse Transcription
- ✧ First-strand cDNA synthesis
- ✧ RNA virus detection(cDNA synthesis)
- ✧ RT-qPCR

II. Contents and Storage

Cat. No.	Contents	Part No.	Quantity
PC026	AccelerRT® III RTase (200U/μl)	PC026-01	50 μl
	5x RT Reaction buffer	PC026-02	200 μl
PC027	AccelerRT® III RTase (200U/μl)	PC026-01	50 μl×5
	5x RT Reaction buffer	PC026-02	200 μl×5

Store all components at -20°C (stable for at least 12 months). Avoid repeated freezing/ thawing.

IV. Procedures

1. Thaw template RNA on ice, Thaw primer, 5x RT Reaction buffer, dNTP Mix, RNase-Free ddH₂O at room temperature. Gently mix reverse transcription reagents and brief centrifugation, then place them on ice immediately.
2. Pre-heat the PCR instrument.
3. Prepare the reaction solution according to the table below. Mix the reaction solution well. Spin down briefly.

Reagent	Volume
RNA template	1pg-1µg total RNA or 1pg-250ng mRNA
50µM Oligo dT18 or	1µL
dNTP Mix(10 mM)	1µL
RNase-Free ddH ₂ O	add to10ul

4. Mix contents thoroughly and spin the tube briefly. Heat mixture to 65°C for 5min and quick chill on ice.
5. Collect the contents of the tube by brief centrifugation and add::

Component	Volume
Mixture 1	10 µL
5x RT Reaction buffer	4µL
AccelerRT® III RTase (200U/µl)	1µL
RNase inhibitor(25U/ul)	1µL
RNase-Free ddH ₂ O	4µL
Total	20µL

6. Mix contents of the tube gently. Incubate at 25°C 10min, 50°C 30min, and inactivate at 85°C for 5min. If you are using Oligo dT and gene specific primer, do not incubate tube at 25°C for 10min.

Note:

1. The solution reagents, vessel, pipettes, centrifuge tubes which used in the experimental reaction should be treated with DEPC H₂O, and then sterilizing. Wearing disposable gloves when you are doing experiments.
2. RNA should be avoided from repeated freeze-thaw, as well as genomic DNA contamination.
3. Collected the contents of the tube by centrifugation before you use the enzyme. You should pipette slowly while pipetting, due to the enzyme high viscosity.
4. The reverse transcription product is used in the next PCR or qPCR experiment, the cDNA volume should not exceed 1/10.

IV. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to the use of **AccelerRT® III Reverse Transcriptase** (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged, or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product, or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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