AccelerRT® 5G Template Switching RT Enzyme Mix

User Manual

Cat. No. **PC020** (10 reactions)
Cat. No. **PC021** (50 reactions)
Cat. No. **PC022** (200 reactions)
Cat. No. **PC023** (1000 reactions)

User Manual

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AccelerRT® 5G Template Switching RT Enzyme Mix

I. Description

AccelerRT® 5G Template Switching RT Enzyme Mix (RNase H-) is a novel RT enzyme that was evolved *in vitro* from MMLV RT. The enzyme possesses RNA- and DNA-dependent polymerase activities but lacks RNase H activity. Its efficient template-switching function allows it to be used for full length cDNA products. The engineered enzyme has greatly improved thermal stability, processability and synthesis rates compared to the wild type MMLV RT enzyme.

■ Feature

a. No RNase H activity.
b. Highly efficient template switching function.
c. Thermostability – 90% active after 60 minutes of incubation at 37-55°C in a reaction mixture.
d. High sensitivity – synthesizes cDNA from a wide range of starting amounts (10 pg ~ 5 µg) of total RNA.
e. High efficiency – completes cDNA synthesis in 15-30 minutes.
f. Enhanced capability to resist RT reaction inhibitors.
g. Incorporates modified nucleotides.

■ Application

- ✓ First-strand cDNA synthesis for full length cDNA products.
- ✓ Construction of single cell sequencing libraries.
- ✓ Discovery and detection of fusion genes.
- ✓ Generation of labeled cDNA probes.
- ✓ RNA analysis by primer extension.

■ Quality Guarantee

No endonuclease, exonuclease, or RNase activity was detected.

II. Contents and Storage

Cat. No.	Contents	Part No.	Quantity
PC020	5G Template Switching RTase Mix	PC020-01	10 µl
	5× 5G RT Reaction buffer	PC020-02	50 µl
PC021	5G Template Switching RTase Mix	PC021-01	50 µl
	5× 5G RT Reaction buffer	PC021-02	200 µl
PC022	5G Template Switching RTase Mix	PC022-01	200 µl
	5× 5G RT Reaction buffer	PC022-02	1 ml
PC023	5G Template Switching RTase Mix	PC023-01	1 ml
	5× 5G RT Reaction buffer	PC022-02	5x 1ml

Store all components at -20°C. Avoid repeated freezing/ thawing.

IV. Procedures

Template Switching function

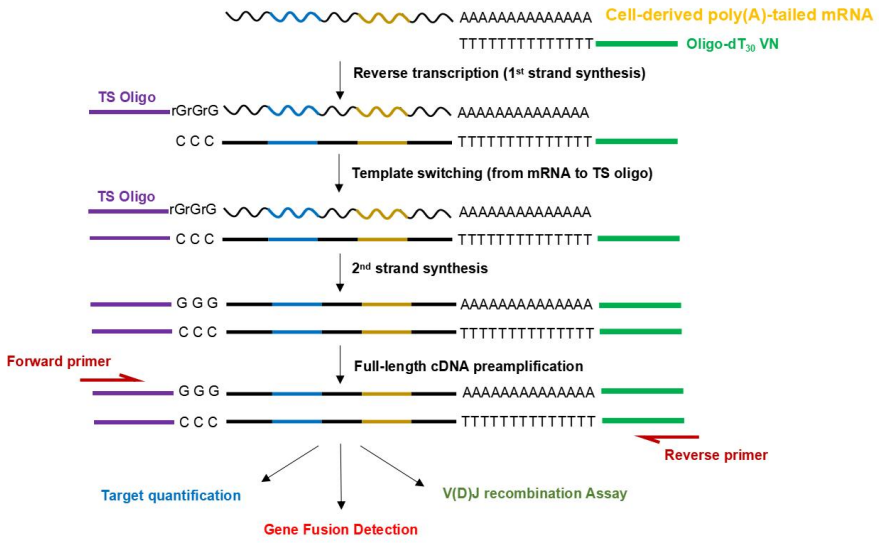

Figure 1. Introduction to the principle of the Template Switching RT Enzyme. The Oligo(dT) VN Primer is used to synthesize the first-strand cDNA. Upon reaching the 5′ end of the RNA template, a few non-template nucleotides are added to the 3' end of cDNA (Template-switching) using the terminal deoxynucleotidyl transferase (TdT) activity of reverse transcriptase. The second-strand of the cDNA is synthesized using a template-switching oligo. Finally, the full-length cDNA product is obtained by PCR amplification using reverse gene-specific primers and forward Template-switching oligo (TSO)-specific primers.

For full-length cDNA synthesis

1. Thaw all the reagents needed for RNA reverse transcription in the AccelerRT® 5G Template Switching RT Enzyme Mix. Mix reagents well by gently inverting the tubes. Spin down briefly and keep on ice.
2. Pre-heat PCR instrument.
3. Prepare RNA reverse transcription reaction solution according to the table below. Mix the reaction solution well. Spin down briefly.

Reagent	Volume	Final concentration
dNTP Mix, 10mM each	1 µl	0.5 mM
10 µM dT primer*1	1 µl	0.5 µM
Total RNA	1ng~ 1 µg	
DEPC H2O	Up to 5 µl	

***1: oligo (dT)20 VN: not suitable for degraded RNA.**

4. 75℃ for 3min, then leave on ice for more than 2 min.
5. Prepare RNA reverse transcription reaction solution according to the table below. Mix the reaction solution well and add to the tube from step 4.

Reagent	Volume	Final concentration
5x 5G RT Buffer	4 µl	1x
10µM TSO*2	2 µl	1 µM
5G Template Switching RTase Mix	1 µl	
RNase Inhibitor (25U/µL)	1 µl	
DEPC H_2O	Up to 15 µl	

***2: Template-switching oligo**

6. The following method for programming the reverse transcription reaction is recommended:

Temperature	Time
42℃	90 min
80℃	10 min
16℃	Hold

Note: The procedures need to be further optimized and adjusted for the different single-cell sequencing platforms based on microfluidics or oil droplet principles.

Regular first strand cDNA synthesis

1. Thaw all the reagents needed for RNA reverse transcription in the AccelerRT® 5G Template Switching RT Enzyme Mix. Mix reagents well by gently inverting the tubes. Spin down briefly and keep on ice.
2. Pre-heat PCR instrument.
3. Prepare RNA reverse transcription reaction solution according to the table below. Mix the reaction solution well. Spin down briefly.

Reagent	Volume	Final concentration
Total RNA	10 pg ~ 5 μg	
dNTP Mix, 10mM each	1 μl	0.5 mM
Random Primer	1 μl	12.5 μM
Oligo $(dT)_{18}$	1 μl	
DEPC H_2O	Up to 10 μl	

4. 65℃ 5 min, then leave on ice for more than 2 min.
5. Prepare RNA reverse transcription reaction solution according to the table below. Mix the reaction solution well and add to the tube of step 4.

Reagent	Volume	Final concentration
5x 5G RT Buffer	4 μl	1x
5G Template Switching RTase Mix	1 μl	
RNase Inhibitor (25U/μL)	1 μl	
DEPC H_2O	Up to 15 μl	

6. The following method for programming the reverse transcription reaction is recommended:

Temperature	Time
25℃*3	10 min
50℃	15~30 min
80℃	10 min
16℃	Hold

***3. If random primer is not added to the reaction system, reverse transcription reaction can be performed directly at 50℃.**

IV. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to the use of AccelerRT® 5G Template Switching RT Enzyme Mix (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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