Ver. 2404-02

HelixCript™ *Thermo* Reverse Transcriptase

Kit Contents

HelixCript™ *Thermo* Reverse Transcriptase		
Cat. No.	**RT10K/RT10KN (10,000 units)**	**RT50K/RT50KN (50,000 units)**
Thermo Reverse Transcriptase (200 units/μl)	0.05ml	0.05ml x 5ea
5x RT Reaction Buffer	0.2ml	0.2ml x 5ea
dNTP Mix (each 10 mM)	None / 0.05ml	None / 0.05ml x 5ea
0.1 M DTT	0.05ml	0.05ml x 5ea
Instructions for Use	1ea	1ea

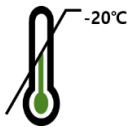
Description

HelixCript™ *Thermo* Reverse Transcriptase, a thermostable and RNase H-negative variant of M-MLV RTase, can synthesize cDNAs from purified RNA templates at a temperature range of 42℃ ~ 55℃ and especially shows the highest activity at 50℃. At the escalated temperature, the cDNA synthesis is enhanced partially due to the less internal structural formation of the template RNA and increased polymerization activity of RTase. The high processivity and productivity of HelixCript™ *Thermo* Reverse Transcriptase allows this enzyme to amplify the high yield of product and can synthesize cDNA of target gene up to 12 kb from RNA template. This enzyme is quite suitable to synthesize the first-strand cDNA for RT-PCR of target gene. The cDNA synthesis from total RNA or poly-(A) RNA is performed by random primer, oligo-d(T) primer, or gene-specific primer.

Application

Generation of first-strand cDNA
RT- PCR, Real-time PCR
Primer extension analysis
RNA sequencing

Storage



Store below -20℃

Shelf life

12 months

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Quality control

By Nanohelix's ISO 13485-certified quality management system, each lot of **HelixCript™ *Thermo* Reverse Transcriptase,** was tested against predetermined specifications to ensure consistent product quality.

Protocol

1. Recommended amounts of RNA template and primers for first-strand cDNA synthesis.

1) RNAs : Total RNA : 10 ng ~ 5 μg
Poly(A)$^+$ RNA : 1 ng ~ 500 ng

2) Primers : Oligo-d(T)$_{20}$: 0.5 μg or 50 pmoles
Random Hexamer : 50 pmoles
Sequence-Specific Primer : 15 ~ 20 pmoles

2. Mix following components in a tube.

Components	Volumes (μl)
RNA Template	X μl
Oligo-d(T), Random hexamer, or Sequence-specific primers (See Protocol 1)	1 μl
dNTP Mix (each 10 mM)	1 μl
RNase-free Water	to 13 μl

3. Incubate at 65℃ for 5 minutes and immediately place on ice.

4. Prepare the reaction mixture by adding the following components in the indicated order.

Adding order	Components	Volume (μl)
1	Template/Primer Mixture (from step 3)	13 μl
2	5x RT Reaction Buffer	4 μl
3	*Thermo* Reverse Transcriptase	1 μl
4	RNase inhibitor (20 unit/ul)	1 μl
5	0.1M DTT	1 μl
Total		**20 μl**

* (Caution) If you do not follow the indicated order, precipitations or loss-of-activity of enzymes will be occurred.

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5. Incubate the reaction mixture at 50℃ for 30 ~ 50 minutes for gene-specific primer.
   When oligo-d(T) or random hexamer is used in reaction, perform 10 min at 42℃, followed by 30 ~ 50 min at 50℃.

6. Inactivate the reaction by heating at 70℃ for 10 min.

7. Synthesized cDNA is immediately used for PCR or store at -20℃.

Products

Cat. No.	Products	Size
RT10K	HelixCript™ *Thermo* Reverse Transcriptase (5x Buffer, 0.1 M DTT)	10,000 units
RT50K	HelixCript™ *Thermo* Reverse Transcriptase (5x Buffer, 0.1 M DTT)	50,000 units
RT10KN	HelixCript™ *Thermo* Reverse Transcriptase (5x Buffer, 0.1 M DTT, dNTPs)	10,000 units
RT50KN	HelixCript™ *Thermo* Reverse Transcriptase (5x Buffer, 0.1 M DTT, dNTPs)	50,000 units

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