Ver. 2502-02

# HelixCript™ 1st-Strand cDNA Synthesis Kit

## Kit Contents

| HelixCript™ 1st-Strand cDNA Synthesis Kit | | | |
|---|---|---|---|
| **Cat. No.** | **CDNA25 (25rxns)** | **CDNA50 (50rxns)** | **CDNA100 (100rxns)** |
| Oligo $d(T)_{20}$ | 0.025ml | 0.05ml | 0.1ml |
| Random Hexamers | 0.025ml | 0.05ml | 0.1ml |
| dNTP Mix (each 10mM) | 0.025ml | 0.05ml | 0.1ml |
| 5x RT Reaction Buffer | 0.1ml | 0.2ml | 0.4ml |
| 0.1M DTT | 0.025ml | 0.05ml | 0.1ml |
| RNase Inhibitor | 0.025ml | 0.05ml | 0.1ml |
| *Thermo* Reverse Transcriptase (200unit/ul) | 0.025ml | 0.05ml | 0.1ml |
| RNase-free Water | 1.0ml | 1.0ml | 1.5ml |
| Instructions for Use | 1ea | 1ea | 1ea |

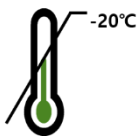
## Description

**HelixCript™ 1st-Strand cDNA Synthesis Kit** is a complete system for efficient synthesis of 1st-strand cDNA from total RNA or poly(A)+-selected RNA. This kit uses HelixCript™ *Thermo* Reverse Transcriptase, a thermostable and RNaseH negative variant of M-MLV RTase, that is active at a temperature range of 42℃ ~ 55℃. 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 included RNase inhibitor in this kit protects the template RNA from degradation in the reaction mix and enhances the yield of cDNA. 1st-strand cDNA synthesized with this kit can be directly used as a template in a downstream assay such as PCR or real-time PCR.

## Application

Generation of first-strand cDNA for subsequent PCR or Real-time PCR
cDNA library construction

**Storage**




Store below -20℃

**Shelf life**

12 months

**NanoHelix Co., Ltd.**

F711-1(Rev.0)

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## Quality Control

Each lot of **HelixCript™ 1st-Strand cDNA Synthesis Kit** 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
Gene-Specific Primer : 15 ~ 20 pmoles

**2.** Prepare the "Template/Primer Mixture" by in a clean and sterile microtube.

| Components | Volumes (μl) |
|---|---|
| RNA Template | Xμl |
| Oligo-d(T)$_{20}$, Random Hexamer, or Gene-Specific Primers | 1μl |
| dNTP Mix (each 10 mM) | 1μl |
| RNase-free Water | Yμl |
| Total | 13μl |

**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) | 13μl |
| 2 | 5x RT Reaction Buffer | 4μl |
| 3 | *Thermo* Reverse Transcriptase (200unit/ul) | 1μl |
| 4 | RNase inhibitor | 1μl |
| 5 | 0.1M DTT | 1μl |
| **Total** | | **20μl** |

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

**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℃.


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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 |
|---|---|---|
| **CDNA25** | HelixCript™ 1st -Strand cDNA Synthesis Kit [All-in-One] | 25rxns |
| **CDNA50** | HelixCript™ 1st -Strand cDNA Synthesis Kit [All-in-One] | 50rxns |
| **CDNA100** | HelixCript™ 1st -Strand cDNA Synthesis Kit [All-in-One] | 100rxns |


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