Cat. # 6215A

For Research Use

TaKaRa

PrimeScript™ IV 1st strand cDNA Synthesis Mix

Product Manual

v201910Da

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I. Description

PrimeScript IV 1st strand cDNA Synthesis Mix is a kit designed for synthesizing 1st strand cDNA from total RNA or polyA$^+$ RNA. It contains a 5X premixed reagent consisting of components required for 1st strand cDNA synthesis (PrimeScript IV Reverse Transcriptase, RNase Inhibitor, Oligo dT Primer, dNTP Mixture, and reaction buffer), and a reaction can be started simply by adding template RNA and water.

The optimized reagent contains an accessory protein that makes it unnecessary to perform RNA denaturation, which is commonly performed before reverse transcription. In addition, PrimeScript IV RTase can synthesize cDNA more rapidly than conventional enzymes. The increased heat resistance of the RTase allows reverse transcription to be performed at a higher temperature, increasing the efficiency and specificity of cDNA synthesis using a gene-specific primer.

The 1st strand cDNA synthesized with this product is suitable for applications involving synthesis of high-quality, full-length cDNA, such as double-stranded cDNA synthesis, hybridization, PCR amplification, preparation of a full-length cDNA library, etc. In addition, using the Random 6 mers included in the kit makes it possible to synthesize cDNA from RNA without polyA$^+$ and to generate cDNA suitable for gene expression analysis by real-time PCR.

II. Components (50 reactions, 20 μl per reaction)

1.	5X PrimeScript IV cDNA Synthesis Mix[*1]	200 μl
2.	Random 6 mers (50 μM)[*2]	100 μl
3.	RNase Free H_2O	1 ml x 2

■ Sequences of individual primers

Name of primer	Sequence
Random 6 mers	pd(N)6
Oligo dT Primer	originally designed by Takara Bio[*3]

*1 Contains PrimeScript IV RTase, RNase Inhibitor, Oligo dT Primer, dNTP Mixture and Reaction Buffer (with Mg^{2+}).

*2 Add to synthesize cDNA from RNA without poly(A) or to perform real-time PCR. This addition makes sure that all regions of RNA are uniformly reverse transcribed.

*3 This is different from the Oligo dT Adaptor Primer in the TaKaRa RNA PCR™ Kit (AMV) Ver. 3.0 (Cat. # RR019A/B) and does not contain the M13 Primer M4 sequence.

Materials Required but not Provided

1. Thermal cycler (or water bath)
2. Micropipettes and pipette tips (autoclaved)

III. Storage -20℃

IV. Protocol: 1st Strand cDNA Synthesis Reaction

[Standard protocol]

1. Prepare the following reaction mixture on ice.

<For one reaction>

Reagent	Volume
5X PrimeScript IV cDNA Synthesis Mix	4 μl
Random 6 mers (50 μM)	≤2 μl [*1,*2]
Template RNA	total RNA: ≤5 μg polyA$^+$ RNA: ≤1 μg
RNase Free H_2O	X μl
Total	20 μl

2. Mix gently and incubate under the following conditions.

30℃ 10 minutes (only when Random 6 mers are added)[*1]
42℃ 10 - 20 minutes [*3]

3. Inactivate the enzyme at 95℃ for 5 minutes or at 70℃ for 15 minutes, and then place the tube on ice.[*4]

*1 We recommend adding 1 to 2 μl of Random 6 mers to synthesize less than 2 kb of cDNA and 0.4 to 1 μl of Random 6 mers to synthesize a 2-kb or longer cDNA. When Random 6 mers are added, perform a preheating step at 30℃ for 10 minutes before the 42℃ incubation.

*2 If a gene specific primer is used, the final primer concentration should be 0.1 μM.

*3 Since PrimeScript IV RTase also shows excellent extensibility for RNA templates with a more complex secondary structure, we recommend performing reverse transcription at 42℃ in a standard reaction. When cDNA is synthesized from more complex RNA templates, especially when using a gene-specific primer as a reverse primer, setting the reverse transcription temperature in the range of 45 to 55℃ may improve results.

*4 After long-length cDNA is synthesized, inactivate the enzyme at 70℃ for 15 minutes in order to avoid damaging the cDNA.

V. RT-PCR

The 1st strand cDNA synthesis reaction mixture can be used directly as a PCR template without purification. However, the volume of the 1st strand reaction must be less than 1/10th of the total PCR reaction volume. Also, for some PCR enzymes, the rate of amplification may be affected by the amount of starting template. Thus, refer to the instructions for the PCR enzyme you are using to determine the appropriate amount of template. In case of nonspecific amplification or PCR that produces no product, the results can be improved by treating the cDNA synthesis reaction with RNase H.

See Section VII. Related Products for recommended PCR enzymes, real-time PCR reagents, and RNase H.

VI. Preparation of RNA Samples

This product is a kit for performing cDNA synthesis from RNA. In order to synthesize cDNA successfully, it is essential to inhibit RNase activity in samples and avoid RNase contamination of equipment and solutions. Additional precautions should be taken during sample preparation, such as using clean disposable gloves and setting aside a designated area exclusively for RNA preparation.

[Equipment]

Disposable plastic equipment should be used whenever possible.

[Solution]

All reagents and purified water should be used exclusively for RNA experiments.

[RNA sample preparation method]

Use of highly purified RNA obtained by the GTC (guanidine thiocyanate) method is recommended. RNA isolation kits such as RNAiso Plus (Cat. #9108/9109)* and NucleoSpin RNA (Cat. # 740955.10/.50/.250)* can also be used for rapid isolation of high purity total RNA. The purified RNA sample should be dissolved in sterile purified water or TE buffer.

* Not available in all geographic locations. Check for availability in your area.

VII. Related Products

<Thermal cycler>

TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. # TP350)*
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. # TP600)*

<Consumables>

0.2 mL single tube
0.2 mL Hi-Tube Dome Cap (Cat. # NJ200)*
0.2 mL Single-Tube Dome Cap (Cat. # NJ204)*
0.2 mL Single-Tube Flat Cap (Cat. # NJ205)*

0.2 mL 8-Strip tubes and caps
0.2 Hi-8-Tube/Dome Cap/Flat Cap (Cat. # NJ300/NJ301/NJ302)*
TaKaRa PCR Micro Strip 8-Tube/Cap (Cat. # 9148/9149)*

<RNA purification>

RNAiso Plus (Cat. # 9108/9109)*
NucleoSpin RNA (Cat. # 740955.10/.50/.250)*
RNase-free Water (Cat. # 9012)
Ribonuclease H (RNase H) (Cat. # 2150A)
Recombinant DNase I (RNase-free) (Cat. # 2270A/B)

<PCR enzyme>

For PCR with a high success rate and high specificity
Tks Gflex™ DNA Polymerase (Cat. # R060A/B)*

For accurate PCR
PrimeSTAR® Max DNA Polymerase (Cat. # R045A/B)
PrimeSTAR® HS DNA Polymerase (Cat. # R010A/B)
PrimeSTAR® GXL DNA Polymerase (Cat. # R050A/B)

For PCR with a high sensitivity and high yield
TaKaRa Ex Taq® (Cat. # RR001A/B)
TaKaRa Ex Taq® Hot Start Version (Cat. # RR006A/B)

For long-chain amplification
TaKaRa LA Taq® (Cat. # RR002A/B)
TaKaRa LA Taq® Hot Start Version (Cat. # RR042A/B)

<Real-time PCR>

Probe qPCR Mix (Cat. # RR391A/B)*
Probe qPCR Mix, with UNG (Cat. # RR392A/B)*
TB Green® Fast qPCR Mix (Cat. # RR430S/A/B)*
TB Green® Premix *Ex Taq ™* II (Tli RNaseH Plus) (Cat. # RR820A/B/L/W)

* Not available in all geographic locations. Check for availability in your area.