Cat. # 6110A

For Research Use

TaKaRa

PrimeScript™ 1st strand cDNA Synthesis Kit

Product Manual

v201903Da

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I. Description

The PrimeScript 1st strand cDNA Synthesis Kit contains all the reagents necessary to synthesize first strand cDNA from total or polyA$^+$ RNA using PrimeScript RTase, a M-MLV (Moloney Murine Leukemia Virus)-derived reverse transcriptase. This enzyme is capable of synthesizing cDNA up to 12 kb in length. PrimeScript RTase can synthesize cDNA efficiently at 42℃, even from RNA templates that contain GC-rich regions or complex secondary structures, making it unnecessary to perform reactions at high temperatures that may degrade RNA. The first strand cDNA synthesized with this kit can be used for variety of applications, including second strand synthesis, hybridization, PCR amplification, and real-time PCR.

II. Components (for 50 reactions)

PrimeScript RTase (200 U/μl)	50 μl
5X PrimeScript Buffer	200 μl
RNase Inhibitor (40 U/μl)	25 μl
dNTP Mixture (10 mM each)	50 μl
Oligo dT Primer (50 μM)	50 μl
Random 6 mers (50 μM)	100 μl
RNase Free dH_2O	1 ml

■ Primer sequences

Primer	Sequence
Random 6 mers	$pd(N)_6$
Oligo dT Primer	a proprietary dT sequence1＊1

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

III. Materials Required by not Provided

1. Water bath
   DNA Amplification System can be used as substitute.
   e.g., TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)＊2
2. Agarose gel
   Agarose L03 [TAKARA] (Cat. #5003), PrimeGel™ Agarose PCR-Sieve (Cat. #5810A), etc.
3. Electrophoresis Apparatus
4. Microcentrifuge
5. Micropipettes and pipette tips (autoclaved)

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

IV. Storage -20℃

V. cDNA Synthesis Protocol

1. Prepare the following mixture in a microtube.

Reagent	Volume
Oligo dT Primer (50 μM)	1 μl
or Random 6 mers (50 μM)	or 1 μl (0.4 - 2 μl)*1
dNTP Mixture (10 mM each)	1 μl
Template RNA	total RNA : < 5 μg*2
	polyA$^+$ RNA : < 1 μg
RNase Free dH$_2$O	x μl
Total	10 μl

2. Incubate for 5 min at 65℃, then cool immediately on ice.*3

3. Prepare the reaction mixture in a total volume of 20 μl.

Reagent	Volume
Template RNA Primer Mixture (from step 2)	10 μl
5X PrimeScript Buffer	4 μl
RNase Inhibitor (40 U/μl)	0.5 μl (20 U)
PrimeScript RTase (200 U/μl)	1.0 μl (200 U)
RNase Free dH$_2$O	x μl
Total	20 μl

4. Mix gently.

5. Incubate the reaction mixture using the following conditions.

30℃	10 min (required when using Random 6 mers)
42℃ (50℃)*4	30 - 60 min

6. Inactivate the enzyme by incubating at 95℃ for 5 min,*5 then cool on ice.

＊1 For Random 6 mers, use 0.4 μl (20 pmol) for synthesis of cDNA products over 2 kb long, and use 2 μl (100 pmol) for reverse transcription prior to real-time PCR. If a gene specific primer is used, use a final primer concentration of 0.1 μM.

＊2 For 1st strand cDNA synthesis of templates for real-time RT-PCR, use less than1 μg of total RNA.

＊3 This denaturing step of template RNA is important to improve the reverse transcription efficiency.

＊4 In general, reactions should be performed at 42℃. However, for RT-PCR reactions where the reverse primer for PCR is also used as a reverse transcription primer, we recommend performing the reverse transcription reaction at 50℃ to reduce the possibility of
non-specific amplification products.

＊5 For synthesis of longer cDNAs, inactivation at 70℃ for 15 min is recommended to minimize cDNA damage (i.e., nicking).

VI. Using cDNA for RT-PCR or Real-time RT-PCR

The first strand cDNA synthesized with this kit can be used directly as a template for PCR or real-time PCR without purification. However, ensure that the cDNA used is less than 1/10 of the total PCR reaction volume. Also, for some PCR enzymes the rate of amplification may be affected by the amount of template. Thus, refer to the PCR enzyme instruction manual to assess the appropriate amount of template to use.
If non-specific amplification or no product is obtained after amplification, results can be improved by treating the cDNA with RNase H.

Recommended PCR enzymes

For excellent, efficient PCR:	*TaKaRa Ex Taq*®, *TaKaRa Ex Taq* HS
For long PCR:	*TaKaRa LA Taq*®, *TaKaRa LA Taq* HS, PrimeSTAR® GXL DNA Polymerase
For accurate PCR:	PrimeSTAR GXL DNA Polymerase, PrimeSTAR Max DNA Polymerase, PrimeSTAR HS DNA Polymerase

Recommended real-time PCR reagents

Intercalator method:	TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus)＊1
	TB Green *Premix Ex Taq* (Tli RNaseH Plus)＊1
Probe method:	*Premix Ex Taq* (Probe qPCR), Probe qPCR Mix＊2

＊1 We have begun the process of changing the names for Takara Bio's intercalator-based real-time PCR (qPCR) products to the "TB Green series".
These products can be used the same way as before, as only the names are changing. Catalog number and product performance are unaffected by this transition.
＊2 Not available in all geographic locations. Check for availability in your area.

VII. Precautions

It is important to use highly pure RNA samples to maximize cDNA yield. It is essential to inhibit cellular RNase activity and also to prevent contamination with RNase derived from equipment and/or solutions. Extra precautions should be taken during sample preparation, including use of clean disposable gloves, dedication of a table exclusively for RNA preparation, and avoiding unnecessary speaking during assembly, to prevent the RNase contamination from sweat or saliva.

[Equipment]

Disposable plastic equipment should be used. Glass tools should be treated with the following protocol prior to use.

(1) Hot-air sterilization (180℃, 60 min)
(2) Treatment with 0.1% diethylpyrocarbonate (DEPC) at 37℃, for 12 hours, followed by autoclaving at 120℃ for 30 min to remove DEPC.

It is recommended that all the equipment be used exclusively for RNA preparation.

[Reagents]

Reagents for RNA preparation, including purified water, should be prepared with heat sterilized glass tools (180℃, 60 min) or, if possible, those treated with 0.1% DEPC solution and autoclaved. Reagents and purified water should be exclusively used for RNA preparation.

[Preparation method]

Simple purification methods can yield enough amount of RNA for reverse transcription and subsequent PCR. However, it is recommended to use highly purified RNA obtained by GTC (Guanidine thiocyanate) method, etc.

VIII. Related Products

PrimeScript™ RT-PCR Kit (Cat. #RR014A/B)*
PrimeScript™ One Step RT-PCR Kit Ver.2 (Cat. #RR055A/B)

TaKaRa Ex Taq® DNA Polymerase (Cat. #RR001A/B)
TaKaRa Ex Taq® DNA Polymerase Hot Start Version (Cat. #RR006A/B)
TaKaRa LA Taq® DNA Polymerase Hot Start Version (Cat. #RR002A/B)
TaKaRa LA Taq® DNA Polymerase Hot Start Version (Cat. #RR042A/B)
PrimeSTAR® DNA Polymerase Hot Start Version (Cat. #R010A/B)
PrimeSTAR® Max DNA Polymerase (Cat. #R045A)
PrimeSTAR® GXL DNA Polymerase (Cat. #R050A/B)

PrimeScript™ RT reagent Kit (Perfect Real Time) (Cat. #RR037A/B)
TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. #RR420A/B)
TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)
Premix Ex Taq™ (Probe qPCR) (Cat. #RR390A/B)
Probe qPCR Mix (Cat. #RR391A/B)*

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

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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