Cat. # 6210A/B

For Research Use

TaKaRa

PrimeScript™ II 1st strand cDNA Synthesis Kit

Product Manual

v201703Da

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I. Description

PrimeScript II 1st strand cDNA Synthesis Kit contains all the reagents necessary to synthesize 1st strand cDNA from total or polyA$^+$ RNA using PrimeScript II RTase. A main factor interfering cDNA synthesis is based on non-specific binding of reverse transcriptase itself to higher structure of RNA in the case of RNA having complex structure or long-length RNA as a template. In addition, non-specific elongation derived from miss-priming of a reverse transcriptase cause a very harmful influence in RT-PCR or production of full-length cDNA. PrimeScript II RTase, which is constructed by further improvement using an accessory protein from PrimeScript RTase developed by Takara Bio, is a reverse transcriptase depressing extremely the harmful factors in the reaction of cDNA synthesis. This kit can synthesize full-length cDNA products efficiently and keep excellent highly efficient of polyA$^+$ RNA usage and synthesis speed at standard temperature (42℃) of reverse transcription in elongation reaction from polyA$^+$ RNA using oligo dT primer. And inhibition of cDNA synthesis does not occur in case that the reaction mixture stand for long time on ice before the reaction of reverse transcription, because this kit suppress completely to occur non-specific elongation in the preparation of reaction mixtures. 1st strand cDNAs synthesized with this kit can be used for variety of applications including 2nd strand synthesis, hybridization, and PCR amplification. Furthermore, this kit is useful for application to construct high-quality, full-length cDNA.

II. Components (for 50 reactions)

PrimeScript II RTase (200 U/μl)	50 μl
5X PrimeScript II 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

■ Sequence of each primer

Primer	Sequence
Random 6 mers	$pd(N)_6$
Oligo dT Primer	Takara Bio originally designed dT sequence ＊

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

■ Reagents and Instrument not supplied in this kit

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

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

III. Storage

-20℃

IV. 1st Strand cDNA Synthesis Reaction

[Standard 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 : less than 5 μg polyA$^+$ RNA : less than 1 μg
RNase Free dH$_2$O	up to 10 μl

2. Keep for 5 minutes at 65℃; cool immediately on ice.
   < Important >
   The efficiency of reverse transcription will increase by denaturation of RNA template.

3. Prepare the reaction mixture by combining the following reagents to a total volume of 20 μl.

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

4. Mix gently.

5. Incubate the reaction mixture immediately under the following conditions.

30℃	10 min (Only when Random 6 mers is used.)
42℃ (- 50℃)＊2	30 - 60 min

6. Inactivate the enzymes by incubation at 95℃ for 5 minutes＊3, followed by cooling on ice.

＊1 For best result, it is recommended to use 0.4 - 1 μl random primers for synthesis of cDNA over 2 kb length, and 1 - 2 μl less than 2 kb length. If a gene specific primer is used, the final primer concentration should be 0.1 μM.

＊2 General reactions should be performed at 42℃ because of PrimeScript II RTase's strong extension against higher-order structures. If using a specific downstream primer for PCR as the reverse transcription primer, there may be some non-specific amplification due to mispriming. In this case, raising the reaction temperature to 45 - 50℃ may improve the result.

＊3 For elongation of longer targets, the inactivation at 70℃ for 15 min. is recommended, so there will be no damage to the 1st strand cDNA (i.e. nicking).

V. RT-PCR

The 1st strand cDNA synthesis reactant can be used directly as 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, there are PCR enzymes for which the rate of amplification may be affected by the amount of starting template. Thus, please refer to PCR enzyme instruction to assess the appropriate amount of template to use.
In case of non-specific amplification or no product produced after PCR, results can be improved by treating the cDNA synthesis reaction with RNase H treatment.

Recommended PCR enzymes

For excellent efficient PCR : *TaKaRa Ex Taq*®, *TaKaRa Ex Taq* Hot Start Version
For long PCR : *TaKaRa LA Taq*®, *TaKaRa LA Taq* Hot Start Version, PrimeSTAR® GXL DNA Polymerase
For Accurate PCR : PrimeSTAR Max DNA Polymerase, PrimeSTAR® GXL DNA Polymerase, PrimeSTAR® HS DNA Polymerase

VI. Preparation of RNA Sample ,

It is important to use highly pure RNA samples for better 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 preparation, to prevent the RNase contamination from user 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.

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

[Reagent]

All reagents to be used in the experiment must be prepared using tools which were treated as described in previous section (Hot-air sterilization (180℃, 60 min) or DEPC treatment), and all purified water must be treated with 0.1% DEPC and autoclaved. All reagents and purified water should be used exclusively for RNA experiments.

[Preparation of RNA sample]

Use of highly purified RNA obtained by GTC (Guanidine thiocyanate) method etc is recommended. The RNA isolation kits such as RNAiso Plus (Cat. #9108/9109) also can be used for isolating high purity of total RNA. The purified RNA sample should be dissolved in sterilized purified water or sterilized TE buffer at process of RNA isolation lastly.

VII. Related Products

For high efficiency, high sensitivity, 2 step RT-PCR for long templates :
PrimeScript® RT-PCR Kit (Cat. #RR014A/B)*

For high sensitivity, 1 step RT-PCR for long templates :
PrimeScript® One Step RT-PCR Kit Ver.2 (Cat. #RR055A/B)

PCR Enzyme

For efficient PCR :
TaKaRa Ex Taq® (Cat. #RR001A/B)
TaKaRa Ex Taq® Hot Start Version (Cat. #RR006A/B)

For long PCR :
TaKaRa LA Taq® (Cat. #RR002A/B)
TaKaRa LA Taq® Hot Start Version (Cat. #RR042A/B)
PrimeSTAR® GXL DNA Polymerase (Cat. #R050A/B)

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

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