PrimeScript 1st strand cDNA Synthesis Kit

The PrimeScript 1st strand cDNA Synthesis Kit contains all of the reagents necessary for synthesis of full-length first-strand cDNA from total or poly A$^+$ RNA, including both oligo(dT) and random 6-mer primers. This kit is powered by PrimeScript Reverse Transcriptase (PrimeScript RT), which has exceptionally strong strand-displacement activity and efficiently synthesizes cDNA. PrimeScript RT is robust, versatile, and well-suited for applications requiring full-length cDNA such as cDNA library construction. This PrimeScript-based cDNA synthesis kit also works well with other cDNA-dependent techniques, including RT-PCR, cDNA probe preparation, and quantitative PCR (qPCR). The PrimeScript 1st strand cDNA Synthesis Kit is ideal for reverse transcription of many different RNA templates, including GC-rich templates and RNAs with high levels of secondary structure.

More

Cat. #	Product	Size	Price	License	Quantity	Details
6110B	PrimeScript™ 1st strand cDNA Synthesis Kit	200 Rxns	$908.00			

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 MMLV (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. Cat. # 6110B contains 4 of Cat. # 6110A. Please refer to Cat. # 6110A for complete product documentation and resources.

Notice to purchaser

Documents	Image Data

6110A	PrimeScript™ 1st strand cDNA Synthesis Kit	50 Rxns	$268.00			

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Overview

- This cDNA synthesis kit includes oligo dT and random 6-mer primers
- Strong strand displacement and extension capability: PrimeScript RT is capable of synthesizing long and full-length cDNA molecules (up to 12 kb)
- High specificity: synthesize cDNA at 42°C—a temperature that does not promote RNA degradation—resulting in low background and higher yields
- Outstanding accuracy: lowest error rate among five commercially available reverse transcriptases tested
- Easy to use: low rates of non-specific annealing, even on incompletely denatured RNA
- Works on challenging templates: excellent results even with templates that are GC-rich or have high levels of secondary structure
- Highly sensitive: use less of your precious RNA samples
- Demonstrated success with RT reaction times of 30 min and 60 min (for longer transcripts)

More Information

Applications

- First-strand cDNA synthesis
- cDNA probe preparation
- RT-PCR
- Synthesis of cDNA libraries with a high proportion of full-length cDNAs

Components

PrimeScript RT (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 H_2O	1 ml

Primer sequences

- Randon 6-mers: pd(N)6
- Oligo dT Primer: Takara Bio proprietary dT sequence region. This sequence is different from the Oligo dT Adaptor Primer supplied with Takara's RNA PCR Kit (AMV) Ver. 3.0. It does not contain the M13 Primer M4 complimentary region.

Standard protocol

1. Prepare the following mixture in a microtube.

Oligo dT Primer (50 µM)*	1 µl
dNTP Mixture (10 mM each)	1 µl
Template RNA	≤5 µg (total RNA) or ≤1 µg (mRNA)
RNase-free dH_2O	to 10 µ

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

2. Heat at 65°C for 5 min, then immediately cool on ice.
3. Prepare the reaction mixture by combining the following components to create a 20-µl reaction.

Template RNA/Primer Mixture	10 µl
5X PrimeScript Buffer	4 µl
RNase Inhibitor	20 Units
PrimeScript RT	100 to 200 Units
RNase-free dH_2O	to 20 µl

4. Mix gently.
5. Perform the reaction under the following conditions.

30°C	10 min*
42 to 50°C**	30 to 60 min

* This step is required for random primers.

** PrimeScript RTase has robust extension capability even with template having complex secondary structure. Therefore, incubation at 42°C is generally recommended. 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°C to reduce the possibility of non-specific amplification products.

6. Heat at 70°C for 15 min and cool on ice.
   The resulting product can be used for a 2nd strand synthesis reaction or as a template for PCR.

Storage

–20°C

Choosing a PrimeScript kit for endpoint RT-PCR

If you want to...	Use this...
Prepare full-length cDNA using your own primers	PrimeScript Reverse Transcriptase (Cat. # 2680A and 2680B)
Prepare full-length cDNA using a kit that includes primers	PrimeScript 1st strand cDNA Synthesis Kit (Cat. # 6110A and 6110B)
Perform 2-step RT-PCR with a kit that includes RT, primers, and PCR enzyme	PrimeScript RT-PCR Kit (Cat. # RR014A and RR014B)
Perform cDNA cloning studies with extremely high fidelity	PrimeScript High Fidelity RT-PCR Kit (Cat. # R022A and R022B)
Perform 1-step PCR	PrimeScript One-Step RT-PCR Kit, Ver. 2 (Cat. # RR055A and RR055B)

Additional product information

Please see the product's Certificate of Analysis for information about storage conditions, product components, and technical specifications. Please see the Kit Components List to determine kit components. Certificates of Analysis and Kit Components Lists are located under the Documents tab.

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