BIO-RAD

iScript™ Select cDNA Synthesis Kit

Catalog #	Description
1708896	**iScript Select cDNA Synthesis Kit**, 25 x 20 µl reactions
1708897	**iScript Select cDNA Synthesis Kit**, 100 x 20 µl reactions
1708897BUN	**iScript Select cDNA Synthesis Kit**, 500 x 20 µl reactions

For research purposes only.

Introduction

The iScript Select cDNA Synthesis Kit is a high-fidelity, sensitive, and flexible kit for the generation of first-strand cDNA. This kit is optimized for reverse transcription PCR (RT-PCR) applications that require high fidelity, including cloning, sequencing, and next-generation sequencing (NGS). It is also optimized for use in reverse transcription quantitative PCR (RT-qPCR) reactions. This robust reverse transcription kit allows a selection of first-strand priming strategies: oligo(dT) primers only, random primers only, or user-designed gene-specific primers.

This cDNA synthesis kit provides all required reagents, except RNA template and gene-specific primers, to create first-strand cDNA from an RNA template. All kit components are optimized to facilitate efficient cDNA synthesis using 1 pg–1 µg input total RNA.

The iScript Reverse Transcriptase contains a recombinant RNase H+ Moloney murine leukemia virus (MMLV) reverse transcriptase preblended with a recombinant RNase inhibitor. The activity of this reverse transcriptase is optimized for use with the 5x iScript Select Reaction Mix. This unique blend of buffers, stabilizers, and dNTPs streamlines reaction setup and ensures robust synthesis of first-strand cDNA. Additionally, the combination of the powerful enzyme and proprietary buffer used in this kit help minimize error rates during cDNA synthesis.

To enhance first-strand priming, the iScript Select cDNA Synthesis Kit incorporates a proprietary enhancer solution into the primer-template hybridization step. To simplify reaction setup, this enhancer is preblended with the oligo(dT) primers and random primers provided in the kit. Consequently, there is no need to add enhancer solution to reactions using the provided primer mixes. However, when using a gene-specific primer (GSP), the enhancer solution must be added to the reaction. A separate protocol and tube labeled GSP enhancer solution is included for this purpose. The addition of enhancer solution to cDNA reactions can significantly improve yields, resulting in earlier detection in real-time PCR.

Storage and Stability

Store at –20°C. Guaranteed for 12 months at –20°C in a constant temperature freezer. Nuclease-free water can be stored at room temperature.

Kit Contents

Reagent	Volume for 25 Reactions	Volume for 100 Reactions	Description
iScript Reverse Transcriptase	25 µl	100 µl	RNase H+ MMLV reverse transcriptase and RNase inhibitor
5x iScript Select Reaction Mix	400 µl	400 µl	5x reaction buffer containing dNTPs, magnesium chloride, and stabilizers
Oligo(dT)$_{20}$ primer mix	200 µl	200 µl	Purified oligo(dT)$_{20}$ primer in a proprietary enhancer solution
Random primer mix	200 µl	200 µl	Purified random primers in a proprietary enhancer solution
GSP enhancer solution	200 µl	200 µl	Proprietary solution for reactions using gene-specific primers
Nuclease-free water	1.5 ml	1.5 ml	

Reaction Setup with Oligo(dT) Primers or Random Primers

Note: This protocol is for use with either oligo(dT) or random primers. Use only the provided primers. Use of primers from other sources can adversely affect performance and sensitivity. For gene-specific primers, follow the protocol in **Reaction Setup with Gene-Specific Primers**. Go to **bio-rad.com/cloning** for more information about using this kit for high-fidelity RT-PCR as part of cloning applications.

1. Thaw all components except iScript Reverse Transcriptase. Mix thoroughly and briefly centrifuge to collect contents to the bottom of each tube before using. Place components on ice.
2. Add the following components to a 0.2 ml PCR tube or each well of a 96-well PCR reaction plate on ice.

Note: For multiple reactions, calculate the amount needed and prepare a master mix with the following components, except RNA, and then dispense to each reaction.

Component	Volume per Reaction, µl
5x iScript Select Reaction Mix	4
iScript Reverse Transcriptase	1
Oligo(dT)$_{20}$ primer or random primer	2
RNA template (1 pg–1 µg total RNA)*	Variable
Nuclease-free water	Variable
Total volume	**20**

* When using larger amounts of input RNA (>1 µg), the reaction should be scaled up (for example, 40 µl reaction for 2 µg or 100 µl reaction for 5 µg) to ensure optimum synthesis efficiency.

3. Mix gently and incubate as follows:
   - For oligo(dT)-primed cDNA reactions, incubate for 10–60 min at 42°C**
   - For random-primed cDNA reactions, incubate for 5 min at 25°C, then 30 min at 42°C
4. Incubate at 85°C for 5 min to heat inactivate the reverse transcriptase.
5. Store cDNA product at –20 to 4°C.
6. The resulting cDNA product can be used directly for PCR amplification. Typically, one-tenth of the first-strand reaction provides sufficient target for most PCR applications. Optionally, the cDNA can be diluted in Tris-EDTA buffer (10 mM Tris [pH 8.0], 0.1 mM EDTA) for addition of larger volumes to PCR reactions.

** For shorter-length targets (1–3 kb), incubation time can be as little as 10 min. For longer (up to 10 kb) or more difficult targets, at least a 60 min incubation is recommended. Incubation time can be extended up to 120 min to synthesize longer cDNAs for cloning purposes.

Reaction Setup with Gene-Specific Primers

Note: This protocol is for use with user-defined gene-specific primers. For oligo(dT) or random primers, follow the protocol in **Reaction Setup with Oligo(dT) Primers or Random Primers**.

1. Thaw all components except iScript Reverse Transcriptase. Mix thoroughly and briefly centrifuge to collect contents to the bottom of each tube before using. Place components on ice.
2. Add the following components to a 0.2 ml PCR tube or each well of a 96-well PCR reaction plate on ice.

Note: For multiple reactions, calculate the amount needed and prepare a master mix with the following components, except RNA, and then dispense to each reaction.

Component	Volume per Reaction, µl
5x iScript Select Reaction Mix	4
iScript Reverse Transcriptase	1
Gene-specific primer (2–10 pmol)	Variable (100–500 nM in 20 µl final volume)
GSP enhancer solution	2
RNA template (1 pg–1 µg total RNA)***	Variable
Nuclease-free water	Variable
Total volume	**20**

*** When using larger amounts of input RNA (>1 µg), the reaction should be scaled up (for example, 40 µl reaction for 2 µg or 100 µl reaction for 5 µg) to ensure optimum synthesis efficiency.

3. Mix gently and incubate at 42°C for 10–60 min.

Note: As required, incubation times can be extended to create longer cDNAs.

4. Incubate at 85°C for 5 min to heat inactivate the reverse transcriptase.
5. Store cDNA product at –20 to 4°C.
6. The resulting cDNA product can be used directly for PCR amplification. Typically, one-tenth of the first-strand reaction provides sufficient target for most PCR applications. Optionally, the cDNA can be diluted in Tris-EDTA buffer (10 mM Tris [pH 8.0], 0.1 mM EDTA) for addition of larger volumes to PCR reactions.

Recommendation for Optimal Results Using the iScript Select cDNA Synthesis Kit

For difficult targets, doubling the volume of iScript Reverse Transcriptase may improve the yield of full-length cDNA. When performing reverse transcription on difficult targets, such as those high in GC content or those with a very long transcript length, preincubation of RNA at 65°C for 5 min followed by incubation on ice can help denature RNA secondary structure and improve the yield of full-length cDNA.

The maximum amount of the cDNA reaction that is recommended for downstream PCR is one-tenth of the reaction volume.

Related Products

Catalog #	Description
Reagents for High-Fidelity PCR	
1725310	iProof™ HF Master Mix
1725320	iProof GC Master Mix
Reagents for Real-Time qPCR	
1725270	SsoAdvanced™ Universal SYBR® Green Supermix
1725280	SsoAdvanced Universal Probes Supermix
1725120	iTaq™ Universal SYBR® Green Supermix
1725130	iTaq Universal Probes Supermix
1725160	SsoAdvanced PreAmp Supermix

Go to **bio-rad.com/PCRReagentSelector** and use our interactive selection tool to find the right reverse transcriptase for your needs. Go to **bio-rad.com/PCRPlasticSelector** and use our interactive selection tool to find the PCR plastics that fit your instrument.

Visit **bio-rad.com/web/selectcDNA** for more information.

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