Reliance Select cDNA Synthesis Kit

Catalog #	Description
12012802	**Reliance Select cDNA Synthesis Kit**, 25 x 20 µl reactions
12012801	**Reliance Select cDNA Synthesis Kit**, 100 x 20 µl reactions

For research purposes only.

Introduction

The Reliance Select cDNA Synthesis Kit is a high-performance reverse transcription (RT) kit that delivers a sensitive and consistent first-strand synthesis of cDNA from challenging sample types. This kit uses Reliance Reverse Transcriptase, an engineered, chimeric enzyme that features high thermostability and inhibitor tolerance and delivers greater sensitivity, fidelity, and data consistency compared to wild-type M-MuLV Reverse Transcriptase.

The Reliance Select cDNA Synthesis Kit provides all required reagents, except gene-specific primers, to create the first-strand cDNA from an RNA template. The flexible kit format allows a selection of priming strategies: oligo(dT) primers only, random primers only, or user-designed gene-specific primers. All kit components are optimized to facilitate efficient cDNA synthesis using a wide range of input RNA (100 fg–2 µg).

Storage and Stability

Reliance Select cDNA Synthesis Kit is guaranteed for 12 months if stored at –20°C in a constant-temperature freezer. When stored under these conditions, kit components (listed in Table 1) are stable for a minimum of 1 year after the shipping date. Nuclease-free water can be stored at room temperature.

Table 1. Kit contents.

Component	Volume for 25 Reactions	Volume for 100 Reactions	Description
5x Reliance Select cDNA Synthesis Reaction Buffer	400 µl	400 µl	5x reaction buffer containing dNTPs, magnesium chloride, and stabilizers
Reliance Reverse Transcriptase	25 µl	100 µl	Chimeric reverse transcriptase with reduced RNase H activity and RNase inhibitor protein
Oligo(dT)$_{20}$ Primer Mix	200 µl	200 µl	Purified oligo(dT)$_{20}$ in a proprietary enhancer solution
Reliance Select Random Primer Mix	200 µl	200 µl	Purified random primers in a proprietary enhancer solution
Nuclease-free water	1.5 ml	1.5 ml	—
DNase	12.5 µl	50 µl	Concentrated custom DNase I solution
DNase buffer	150 µl	150 µl	Concentrated proprietary DNase buffer solution

Reaction Setup for Removing Genomic DNA

For optimal results, reactions should be assembled on ice using sterile and nuclease-free tubes, tube strips, or plates. Contamination by genomic DNA (gDNA) carryover in RNA samples can be tested using a no-RT control.

1. Prepare a DNase master mix by combining 0.5 µl DNase and 1.5 µl DNase buffer per reaction. Please note that the kit component volumes provided do not take into account the preparation of excess master mix. Mix thoroughly by pipetting up and down several times.
2. Add 2 µl of the DNase master mix to each 11 µl RNA sample or diluted RNA + water sample for a total reaction volume of 13 µl. Mix thoroughly by pipetting up and down several times.

   Note: Input RNA amounts, ranging from 100 fg to 2 µg, must be optimized based on target gene abundance and sample availability. Ensuring the quality and purity of the RNA sample is essential for achieving the highest capacity of cDNA.
3. Using a thermal cycler with the heated lid on, incubate the reaction(s) according to the guidelines in Table 2.

Table 2. DNase reaction protocol.

Step	Temperature, °C	Time, min
DNA digestion	25	5
DNase inactivation	75	5

Note: To avoid damage to the RNA, proceed directly to cDNA synthesis. However, if longer storage is necessary, store the treated RNA at –80°C or on dry ice and thaw on ice when proceeding with the cDNA synthesis reaction.

Reaction Setup for cDNA Synthesis with Oligo(dT) Primers or Random Primers

Important Note — Please Read Before Starting

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. To use gene-specific primers, follow the guidelines in the Reaction Setup for cDNA Synthesis with Gene-Specific Primers section.

1. Thaw all components except Reliance Reverse Transcriptase. Mix thoroughly and briefly centrifuge to collect contents at the bottom of the tube before using. Place components on ice.
2. Prepare the reactions according to Table 3 by adding the following components to a 0.2 ml PCR tube or each well of a 96-well PCR reaction plate on ice.

Table 3. Reaction setup with oligo(dT) primers or random primers.

Component	Volume per 20 µl Reaction
5x Reliance Select cDNA Synthesis Reaction Buffer	4 µl
10x oligo(dT) primer mix or random primer mix	2 µl
RNA template	Variable (100 fg–2 µg)
Reliance Reverse Transcriptase	1 µl
Nuclease-free water	Variable
Total reaction mix volume	**20 µl**

Note: For multiple reactions, prepare a master mix with the above components, except RNA template, and dispense into reaction vessels.

3. Mix gently and incubate as follows:
   - For **oligo(dT)-primed cDNA reactions**, incubate at 50°C for 60–90 min
   - For **random-primed cDNA reactions**, incubate at 50°C for 20 min
4. Incubate at 95°C for 1 min to heat inactivate the reverse transcriptase.
5. Store cDNA product at –20 to 4°C.

The resulting cDNA product can be used directly for PCR amplification. Typically, one-tenth (2 µl) of the first-strand reaction provides sufficient target for most PCR applications. Depending on target abundance, cDNA libraries prepared using 1 µg or more of input RNA may need to be diluted at least tenfold in TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) or nuclease-free water prior to use in quantitative PCR (qPCR). Optionally, the cDNA can be diluted in TE buffer for addition of larger volumes (5–10 µl) to PCR reactions.

Reaction Setup for cDNA Synthesis with Gene-Specific Primers

Important Note — Please Read Before Starting

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

1. Thaw all components except Reliance Reverse Transcriptase. Mix thoroughly and briefly centrifuge to collect contents to the bottom of the tube before using. Place the components on ice.
2. Prepare the reactions according to Table 4 by adding the following components to a 0.2 ml PCR tube or each well of a 96-well PCR reaction plate on ice.

Table 4. Reaction setup with gene-specific primers.

Component	Volume per 20 µl Reaction
5x Reliance Select cDNA Synthesis Reaction Buffer	4 µl
Gene-specific primers (2–10 pmol)	Variable (100–500 nM in 20 µl final volume)
RNA template	Variable (100 fg–2 µg)
Reliance Reverse Transcriptase	1 µl
Nuclease-free water	Variable
Total reaction mix volume	**20 µl**

Note: For multiple reactions, prepare a master mix with the above components, except RNA template, and dispense into reaction vessels.

3. Mix gently and incubate at 50°C for 10–60 min.

Note: Incubation times can be extended to synthesize longer cDNAs for cloning purposes.

4. Incubate at 95°C for 1 min to heat inactivate the reverse transcriptase.
5. Store cDNA product at –20 to 4°C.

The resulting cDNA product can be used directly for PCR amplification. Typically, one-tenth (2 µl) of the first-strand reaction provides sufficient target for most PCR applications. Optionally, the cDNA can be diluted in TE buffer for addition of larger volumes (5–10 µl) to PCR reactions.

Recommendations for Optimal Results

The maximum amount of cDNA reaction recommended for downstream PCR is one-tenth of the reaction volume, typically 2 µl.

Control Reactions

Negative control reactions should be included in any experiment to verify the results of the first-strand cDNA synthesis.

No Reverse Transcriptase Control Reaction

To confirm the absence of contaminating gDNA in an RNA sample, the no-RT control reaction should contain every reagent for the reverse transcription reaction except for the Reliance Reverse Transcriptase.

- If no gDNA contamination is present, this reaction should yield a negative amplification result
- If gDNA is present in the RNA sample, pretreat the RNA sample with DNase before cDNA synthesis

No Template Control (NTC) Reaction

To confirm the absence of reagent contamination, the NTC reaction should contain all reagents for the reverse transcription reaction except for the RNA template. The NTC reaction should yield a negative amplification result.

Quality Control

Reliance Select cDNA Synthesis Kit demonstrates high-RT efficiency and linear resolution over a wide linear dynamic range. Stringent specifications are maintained to ensure lot-to-lot consistency. This product is free of detectable DNase and RNase activities.

Related Products

Catalog #	Description
Cell Lysis RT-qPCR Kit	
1725080	**SingleShot Cell Lysis Kit**
Reagents for Real-Time PCR	
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**

Visit **bio-rad.com/qPCR** for more information about real-time PCR systems and analysis software.

Visit **bio-rad.com/pcrplastics** for a diverse selection of PCR plastics and seals.

Visit **bio-rad.com/PrimePCR** to view a full range of qPCR gene expression and long noncoding RNA PrimePCR Assays.

Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Drive, Hercules, CA 94547
510-741-1000

10000124806 Ver B 21-0681 0921