Lucigen®

Simplifying Genomics

EpiScript™ RNase H⁻ Reverse Transcriptase

Cat. Nos. ERT12910K and ERT12925K

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1. Introduction

EpiScript™ RNase H⁻ Reverse Transcriptase (EpiScript RT), an alternative to SuperScript® II Reverse Transcriptase, is a recombinant MMLV reverse transcriptase with reduced RNase H activity. It is highly efficient at producing full-length cDNA from long RNA templates. EpiScript RT demonstrates significantly more activity than other MMLV Reverse transcriptase enzymes. It is capable of producing cDNA from as little as 100 pg of total RNA for real-time RT-PCR (qRT-PCR) analysis and other applications.

EpiScript RT is supplied at a concentration of 200 U/µL. A 10X RT Reaction Buffer and 100 mM dithiothreitol (DTT) are also provided.

2. Product Designations and Kit Components

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
EpiScript RNase H⁻ Reverse Transcriptase	10,000 Units	ERT12910K	EpiScript Reverse Transcriptase (200 U/µL)	E0144-200D1	50 µL
			10X RT Reaction Buffer	SS000737-D2	250 µL
			DTT (100 mM)	SS000065-D6	250 µL
EpiScript RNase H⁻ Reverse Transcriptase	25,000 Units	ERT12925K	EpiScript Reverse Transcriptase (200U/µL)	E0144-200D2	125 µL
			10X RT Reaction Buffer	SS000737-D3	600 µL
			DTT (100 mM)	SS000065-D8	600 µL

3. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: EpiScript RT is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM sodium chloride, 1 mM DTT, 0.1 mM EDTA, and 0.1% Triton® X-100.

Unit Definition: One unit of EpiScript RT catalyzes the incorporation of 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C using saturating amounts of oligo(dT)-primed poly(rA) as template.

Quality Control: EpiScript RT is function-tested in a control reaction using an oligo(dT) primer. In this reaction, the enzyme converts 200 ng of ~2 kb poly(A) RNA into full-length cDNA in 30 minutes or less at 37°C.

Contaminating Activity Assays: All EpiScript RT components are free of detectable contaminating DNA exonuclease and endonuclease, and RNase activities.

4. General Considerations for cDNA Synthesis

Maintaining an RNase-free Environment:

Ribonuclease contamination is a significant concern for those working with RNA. The ubiquitous RNase A is a highly stable and active ribonuclease that can contaminate any lab environment and is present on human skin. However, creating an RNase-free work environment and maintaining RNase-free solutions is critical for performing successful cDNA synthesis reactions. Therefore, we strongly recommend that the user:

1) Autoclave all tubes and pipette tips that will be used in the cDNA synthesis reactions.
2) Always wear gloves when handling samples containing RNA. Change gloves frequently especially after touching potential sources of RNase contamination such as door knobs, pens, pencils, and human skin.
3) Always wear gloves when handling kit components. Do not pick up any kit component with an ungloved hand.
4) Keep all kit components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps.

We *strongly* recommend the addition of Lucigen's RiboGuard™ RNase Inhibitor to each reaction.

Choice of Primer for First-Strand cDNA Synthesis:

First-strand cDNA synthesis can be primed using either an **oligo(dT) primer, random primers**, or **gene-specific** primers (all primers to be provided by the user).

An **Oligo(dT) primer** is the most commonly used method for priming first-strand cDNA synthesis when using an eukaryotic RNA sample. Oligo(dT) primes cDNA synthesis only from the poly(A) tail present at the 3′ end of almost all eukaryotic mRNAs. Since poly(A) RNA constitutes just 1-5% of the RNA in a eukaryotic total cellular RNA preparation, the complexity of the cDNA produced is significantly less than when the cDNA is synthesized using random primers. Lower-complexity cDNA can result in a more sensitive and specific PCR amplification. Additionally, priming cDNA synthesis with an oligo(dT) primer precludes the need to enrich the RNA sample for poly(A) RNA. Typically, the oligo(dT) primer is 18-21 nucleotides in length.

Random primers initiate cDNA synthesis from all RNA species (rRNA and mRNA) in a total cellular RNA sample. Since rRNA, which constitutes >95% of the RNA in a total RNA sample, is converted to cDNA using random primers, the complexity of the cDNA will be much greater than when priming the reaction with oligo(dT). As a result, random primers are much less frequently used than oligo(dT) primers. Random primers are typically 6-9 nucleotides in length and can be helpful when:

1) Synthesizing cDNA from mRNAs that lack a poly(A) tail (such as bacterial mRNA) or have a very short poly(A) tail;
2) Priming cDNA synthesis from partially degraded RNA samples such as those obtained from formalin-fixed paraffin-embedded (FFPE) tissue samples;
3) Priming cDNA synthesis of a poly(A)-enriched RNA sample;
4) It is necessary to eliminate or reduce 3′ sequence bias that can result when using an oligo(dT) primer.

Gene-specific primers, designed and synthesized by the user, provide the greatest specificity when priming cDNA synthesis of an mRNA. However, the user frequently must determine the optimal primer annealing and extension (reverse transcription) conditions empirically for each primer used.

5. EpiScript™ RNase H⁻ Reverse Transcriptase cDNA Synthesis Procedure

The following protocol has been optimized to convert 100 pg of cellular RNA to 1 µg of first-strand cDNA using an oligo(dT) primer or random primers. The use of gene-specific primers may require additional optimization of the reaction.

Gently mix and briefly centrifuge all kit components prior to dispensing.

1. Denature the RNA sample and anneal the primer(s). For each reaction, combine the following components on ice, in a sterile (RNase-free) 0.2-mL or 0.5-mL tube:

x	µL	RNase-Free Water
x	µL	Total RNA sample (up to 1 µg)
10	pmol	$Oligo(dT)_{18\text{-}21}$ primer
		- or -
100	ng	Random primers
		- or -
x	µL	Gene-specific primers
10	µL	Total reaction volume

2. Incubate at 65°C for 2 minutes in a water bath or thermocycler with heated lid.
3. Chill on ice for 1 minute. Centrifuge briefly in a microcentrifuge.
4. For each reaction, combine the following components on ice:

x	µL	RNase-Free Water	
10	µL	Annealed RNA:primer(s) (from step 3 above)	
2	µL	10X RT Reaction Buffer	
2	µL	100 mM DTT	
0.5	µL	RiboGuard RNase Inhibitor (optional, sold separately)	
1	µL	10 mM dATP	Solution (provided by the user, sold separately)
1	µL	10 mM dCTP	Solution (provided by the user, sold separately)
1	µL	10 mM dGTP	Solution (provided by the user, sold separately)
1	µL	10 mM dTTP	Solution (provided by the user, sold separately)
0.5	µL	EpiScript Reverse Transcriptase (200 U/µL)	
20	µL	Total reaction volume	

5. Mix the reaction gently.

   If using oligo(dT) primers, incubate the reaction at 37°C for 60 minutes.

   If using random primers, incubate the reaction at room temperature for 10 minutes and then at 37°C for 60 minutes.
6. Terminate the reaction by heating at 85°C for 5 minutes.
7. Chill on ice for at least 1 minute. Centrifuge briefly in a microcentrifuge.
8. The cDNA can be used immediately, without purification, for end-point or real-time PCR (qPCR), converted to double-stranded cDNA, or stored at –20°C for future use.