Ultra Script II Reverse Transcriptase

Cat: PE5012 **Size: 2000 Units**
I Store at -20°C.

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Ultra Script II Reverse Transcriptase is stable for 2 year when stored at -20°C (non-frost-free).

Description
Ultra Script II Reverse Transcriptase (RNaseH Minus) is an engineered from Moloney Murine Leukemia Virus RT to reduce RNaseH activity and enhance cDNA yield. RnaUsScript RT is a single polypeptide chain with a molecular weight of approximately 78 kDa, is an RNA-dependent DNA polymerase that synthesizes the complementary cDNA strand from a single-stranded RNA template to which a primer has been annealed (1,2). RnaUsScript RT also efficiently extend primers hybridized to single-stranded DNA. This enzyme is expressed in *E. coli* (2) and purified to free of exogenous RNase activity, a common contaminant in commercial preparations of reverse transcriptase.The enzyme is used to synthesize first-strand cDNA up to 12 kb and 5X cDNA Synthesis buffer is provided.

Components

	PE5012 (2,000 Units)
• Ultra Script II Reverse Transcriptase	10 µl
• 5X cDNA Synthesis Buffer {250 mM Tris-HCl(pH 8.3 at 25°C), 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT, and enhancer}	0.4 ml

Product Qualification
Ultra Script II Reverse Transcriptase is functionally tested for amplification of a 530-bp of GAPDH mRNA target with 100pg of total HeLa RNA.

Product Specifications
Storage Buffer: 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) NP40S, 50% (v/v) glycerol
Unit Definition: One unit of MMLV RT catalyzes the incorporation of 1 nmol of dTTP into acid- insoluble material in 10 minutes at 37°C using oligo$(dT)_{12\text{-}18}$-primed poly(A)n as a template.
Contaminant Activity: LeGene RnaUsScript RT product is free of exogenous RNase and endonuclease when measured by RNA ladder and supercoiled plasmid DNA degradation method.
Purity: The purity is >95% as judged by SDS-PAGE gels with Comassie blue staining.

Recommended 1st Strand cDNA Synthesis Reaction Protocol
1 Prepare RNA/Primer mixtures
a). Using Oligo $(dT)_{20}$

Components	**1X Rxn**
RNA (10 pg- 5 µg)	x µl
Oligo $(dT)_{20}$ (50 µM)	1.0 µl
10 mM dNTPs	1.0 µl
Nuclease-free water	to 10 µl

(b). Using Gene Specific Primer (GSP)

Components	**1X Rxn**
RNA (10 pg- 5 µg)	x µl
GSP (2 µM)	1.0 µl
10 mM dNTPs	1.0 µl
Nuclease-free water	to 10 µl

(c). Using Random Hexamer

Components	1X Rxn
RNA (10pg- 5 μg)	x μl
Random Hexamer (50 ng/μl)	1.0 μl
10 mM dNTPs	1.0 μl
Nuclease-free water	to 10 μl

2. Incubate the above sample at 65 °C for 3 minutes and place on ice until use.
3. Prepare the following RT reaction mixture.

Components	1X Rxn	10X Rxn
5X cDNA Synthesis Buffer	4.0 μl	40 μl
RnaUsScript RT (200 units)	0.25-1.0 μl	2.5-10 μl
Nuclease-free water	5.0 μl	50 μl
Total	10 μl	100 μl

**Note*: 50-200 units of RnaUsScript RT per reaction is recommended. When using less than 100 pg of starting RNA, the addition of RNase Inhibitor is recommended

4. Add 10 μl of reaction mixture to each RNA/Primer mix, mix gently and then centrifuge 3 seconds to collect contents..
5. The following RT temperature and time is recommended.
   - Oligo$(dT)_{20}$ and GSP based cDNA synthesis: Incubate at 45°C for 50 minutes
   - Random Hexamer based cDNA synthesis: Incubate at room temperature (22-24°C) for 5 minutes then transfer the tubes to 45°C and incubate for 50 minutes.

* For mRNA targets <2kb, 30 minutes incubation at 45°C is an optional choice. Also, it can be raise RT reaction temperature up to 50°C if needed.

6. Inactivate RT by heating at 85°C for 5 min
7. Use 1-2 μl of the first strand reaction out of 20 μl for PCR amplification. Store the remaining first strand cDNA sample at -20 °C until use.

Recommended PCR Reaction Protocol

The following protocol is suggested as a starting point.

Components	25 ul Rxn	50 ul Rxn	Final Concentration
10X PCR Buffer	2.5 μl	5.0 μl	1X
50 mM $MgCl_2$	0.75 μl	1.5 μl	1.5 mM (Variable:1.0-2.0 mM)
10 mM dNTPs (dA/dC/dT/dGTP)	0.5 μl	1.0 μl	0.2 mM
DnaUs *Taq* DNA Polymerase (5U/μl)	0.1-0.2 μl	0.2 μl	0.5-1.0 Unit
Forward Primer (10 μM)	0.5 μl	1.0 μl	200 nM (Variable:100-500 nM)
Reverse Primer (10 μM)	0.5 μl	1.0 μl	200 nM (Variable:100-500 nM)
Template DNA	x μl	x μl	Variable (fg -μg)
Final Volume (μl)	25 μl	50 μl	

1. Assemble the reaction on ice.
2. Cap reaction vessels and load in thermal cycler at 94°C.
3. Incubate tubes in a thermal cycler at 94°C for 1 min to completely denature the template.
4. Perform 25-40 cycles of PCR amplification as follows:
   - Denature 94°C for 15–30 s
   - Anneal 55-60°C for 15–30 s
   - Extend 72°C for 1 min per kb
   - Hold at 4°C until use
5. Analyze PCR products by gel electrophoresis.

Other Applications:

Ultra Script II Reverse Transcriptase can use for RT-PCR, end-labeling of DNA, mRNA 5'-end mapping by primer extension analysis, dideoxynucleotide sequencing, synthesis of radioactive cDNA probes(3).

References:

1. Gerard, G.F. and Grandgenett, D.P. (1975) *J. Virology* 15, 785.
2. Roth, M.J. *et al*., (1985) *J. Biol. Chem*. 260, 9326.
3. Sambrook, J. *et al*., (1989) *Molecular Cloning: A Laboratory Manual (2nd ed.)* New York: Cold Spring Harbor Laboratory Press.

Limitations of Use
For research use only. Not for use in diagnostic procedures.