ABScript III Reverse Transcriptase

Catalog: RK20408
Size: 4,000 U / 10,000 U
Concentration: 200,000 U/mL
Components:

ABScript III Reverse Transcriptase (200,000 U/mL)	RM21402
5X First-Strand Reaction Buffer	RM20109
100 mM DTT (10X)	RM20117

Product Description

ABScript III Reverse Transcriptase is a genetic-modified M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active up to 48°C, which provides higher specificity and higher yield of cDNA.. The first strand cDNA product generated is up to 12kb.

Product Souce

An *E. coli* strain that carries the cloned modified *M-MuLV* Reverse Transcriptase (RNase H-) gene.

Unit Definition

One unit of ABScript III Reverse Transcriptase is the amount of enzyme required to incorporate 1 nmole of deoxyribonucleotide into acid-precipitable material in 10 min. at 37°C using poly(A)•oligo(dT)12-18 as template•primer

Stocking buffer

20 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.01% IGEPAL® CA-630, pH 7.5 @ 25°C

Storage temperature

-20°C

1X First-Strand Reaction Buffer

50 mM Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$, pH 8.3 @ 25°C

Inactivation

85°C for 20 min

First Strand cDNA Synthesis Protocols

➢ Easy protocal

Thaw kit components on ice and mix by inverting several times.

- Mix the following components and incubate at 50°C for 1 hour. If Random Primer Mix is used, an incubation step at 25°C for 5 minutes is recommended before the 42°C incubation.

	1 × RXN
ddH_2O	Up to 20 µL
5X First-Strand Buffer	4 µL
Template RNA	Up to 1 µg*
50 µM $d(T)_{23}VN$ or 60 µM Random Primer	2 µL
10 mM dNTPs	1 µL
100 mM DTT	2 µL
RNase Inhibitor (40 U/µL)	0.2 µL (8 U)
ABScript III Reverse Transcriptase**	1 µL (200 U)

**: 1 ng-1 µg whole RNA or 50 pg-100 ng Poly(A)-RNA.*

***: ABScript III Reverse Transcriptase is active at 50-55°C.*

- Inactivate the enzyme at 85°C for 5 minutes. For downstream PCR application, the volume of cDNA product should not exceed 1/10 of the PCR reaction volume.

➢ Standard Protocol

If denaturation of template RNA is desired, use the following protocol.

- Mix RNA sample and primer $d(T)_{23}VN$ in a sterile RNase-free microfuge tube.

	1 × RXN
Template RNA	Up to 1 μg*
50 μM $d(T)_{23}VN$ or 60 μM Random Primer	2 μL
10 mM dNTPs	1 μL
ddH_2O	Up to 10 μL

**: 1 ng-1 μg whole RNA or 50 pg-100 ng Poly(A)-RNA.*

- Denature sample RNA/$d(T)_{23}VN$ for 5 minutes at 65℃. Spin briefly and put promptly on ice.
- Add the following components

	1 × RXN
ddH_2O	Up to 20 μL
Denatured RNA/primers mix of last step	10 μL
5X First-Strand Buffer	4 μL
100 mM DTT	2 μL
RNase Inhibitor (40 U/μL)	0.2 μL
ABScript III Reverse Transcriptase	1 μL

- Incubate the 20 μl cDNA synthesis reaction at 50℃ for one hour. If Random Primer Mix is used, an incubation step at 25℃ for 5 minutes is recommended before the 42℃ incubation.

NOTE: ABScript III Reverse Transcriptases is active at 50-55℃.

- Inactivate the enzyme at 85℃ for 5 minutes. The cDNA product should be stored at -20°C. In general, the volume of cDNA product should not exceed 1/10 of the PCR reaction volume.

➢ No-RT Negative Control Reaction

Mix the following components and incubate at 50℃ for 1 hour.

	1 × RXN
ddH_2O	Up to 20 μL
5X First-Strand Buffer	4 μL
Template RNA	Up to 1 μg*
50 μM d(T)23VN or 60 μM Random Primer	2 μL
10 mM dNTPs	1 μL
100 mM DTT	2 μL
RNase Inhibitor (40 U/μL)	0.2 μL (8 U)

**: 1 ng-1 μg whole RNA or 50 pg-100 ng Poly(A)-RNA.*

***: ABScript III Reverse Transcriptase is active at 50-55℃.*

Quality Controls

- ◆ Enzyme concentration is above 95% by testing from SDS-PAGE.
- ◆ Free of detectable DNA exonuclease and endonuclease.
- ◆ No residual host genomic DNA detected by PCR.

Related Product

RK21401, RNase Inhibitor, Mammalian (40,000 U/mL)