ABScript II Reverse Transcriptase

Catalog: RK21400
Size: 4,000 U / 10,000 U
Concentration: 200,000 U/ml
Components:

ABScript II Reverse Transcriptase (200,000 U/ml)	RM21400
5X First-Strand Buffer	RM20109
100 mM DTT (10X)	RM20117

Product Description

ABScript II Reverse Transcriptase is a recombinant 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, providing higher specificity, higher yield of cDNA, and more full-length cDNA product up to 12 kb.

Product Source: The gene encoding a mutant M-MuLV Reverse Transcriptase (RNase H^-) is expressed in *E. coli* and purified to near homogeneity.
Unit Definition: One unit is defined as the amount of enzyme that incorporates 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C using poly(rA)•oligo$(dT)_{18}$ as template, with a total reaction volume of 50 µl.

Reaction Conditions:
1X First-Strand Reaction Buffer, 10 mM DTT, 200 units ABScript II Reverse Transcriptase, supplemented with 0.5 mM dNTPs (not included) and 5 µM $dT_{23}VN$ (not included). Incubate at 42°C for 50 minutes. If random primers are used, a 10-minute incubation at room temperature is recommended before transferring to 42°C.

1X First-Strand Reaction Buffer:
50 mM Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$, pH 8.3 @ 25°C

Storage Temperature: -20°C
Storage Conditions:
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

Heat Inactivation: 65°C for 20 min

Instructions

➢ **First Strand cDNA Synthesis (Quick Protocol)**

Thaw components on ice and mix by inverting several times.

1. Mix the following components and incubate at 42°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.

COMPONENT	VOLUME
Nuclease-free H_2O	Up to 20 µl
5X First-Strand Buffer	4 µl
$d(T)_{23}VN$ (50 µM) or Random Primer Mix (60 µM)	2 µl
100 mM DTT	2 µl
RNase Inhibitor (40 U/µl)	0.2 µl
Template RNA	up to 1 µg*
10 mM dNTP	1 µl
ABScript II RT (200 U/µl)	1 µl

* 1 ng-1 µg total RNA or 50 pg-100 ng poly(A)-RNA
** ABScript II Reverse Transcriptase can be used at 42–48°C.

2. Inactivate the enzyme at 65°C for 20 minutes. For downstream PCR application, the volume of cDNA product should not exceed 1/10 of the PCR reaction volume.

➢ **First Strand cDNA Synthesis (Standard Protocol)**

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

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

COMPONENT	VOLUME
Total RNA	up to 1 µg*
$d(T)_{23}VN$ (50 µM) or Random Primer Mix (60 µM)	2 µl
10 mM dNTP	1 µl

Nuclease-free H_2O	Up To 10 µl

2. Denature sample RNA/primer for 5 minutes at 65°C. Spin briefly and put on ice immediately.

3. Add the following components to the tube

COMPONENT	VOLUME
Nuclease-free H_2O	Two Steps Total Up to 20 µl
5X First-Strand Buffer	4 µl
100 mM DTT	2 µl
RNase Inhibitor (40 U/µl)	0.2 µl
ABScript II RT (200 U/µl)	1 µl

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

5. Inactivate the enzyme at 65°C for 20 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.

* 1 ng-1 µg total RNA or 50 pg-100 ng poly(A)-RNA

** ABScript II Reverse Transcriptase can be used at 42–48°C.

➢ First-strand cDNA Synthesis (No-RT Negative Control Reaction)

1. Mix the following components and incubate at 42°C for 1 hour:

COMPONENT	VOLUME
Template RNA	up to 1 µg*
$d(T)_{23}VN$ (50 µM) or Random Primer Mix (60 µM)	2 µl
5X First-Strand Buffer	4 µl
100 mM DTT	2 µl
10 mM dNTP Mix	1 µl
RNase Inhibitor (40 U/µl)	0.2 µl
Nuclease-free H2O	Up to 20 µl

* 1 ng-1 µg total RNA or 50 pg-100 ng poly(A)-RNA

Additional Products:

RNase Inhibitor (40,000 U/mL) is available separately (Cat. NO. RK20401).

QC Process:

- ◆ Purity is above 95% detected by SDS-PAGE.
- ◆ No exonuclease, nuclease, RNase contamination.
- ◆ Host genomic DNA is no residual detected by PCR.