First Strand cDNA Synthesis Protocols (E6560)

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

Easy Protocol

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
Template RNA	up to 1 µg
$d(T)_{23}$ VN	2 µl
ProtoScript II Reaction Mix (2X)	10 µl
ProtoScript II Enzyme Mix (10X)	2 µl
Nuclease-free H_2O	to a total volume of 20 µl

2. Inactivate the enzyme at 80°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 denature 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	1–6 µl (up to 1 µg)
$d(T)_{23}$ VN (50 µM)	2 µl
Nuclease-free H_2O	to a total volume of 8 µl

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

ProtoScript II Reaction Mix (2X)	10 µl
ProtoScript II Enzyme Mix (10X)	2 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 80°C 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 42°C for 1 hour.

COMPONENT	VOLUME
Total RNA	up to 1 µg
$d(T)_{23}$ VN (50 µM)	2 µl
ProtoScript II Reaction Mix (2X)	10 µll
Nuclease-free H_2O	to a total volume of 20 µl