

Manual

Product name: SmartRT™ Reverse Transcriptase

Cat #: SMRT-100, SMRT-200, SMRT-300, SMRT-OEM

Description:

SmartRT™ Reverse Transcriptase is an engineered MMLV RT with improved enzyme thermostability, cDNA synthesis ability, and reduced RNase H activity. The enzyme also has a terminal transferase activity where it adds a few extra nucleotides to the end of the synthesized cDNA. With a 3' modified oligo dT primer and 5' SMART universal oligo containing a terminal complementation to nuclear acids at 3' end of the first-strand cDNA, the SmartRT Reverse Transcriptase will produce RACE ready full length cDNA.

Features:

Our reverse transcriptase is one of the transcriptases that has the highest thermostability with terminal transferase activity.

Recommended Storage Condition: -20 °C

Protocol:

For RACE ready first-strand cDNA Synthesis

Combine the following in separate 0.2-ml PCR tubes (20 µl reaction):

- 1 µg RNA sample
- 1 µl 5' SMART Universal Primer
- 1 µl oligo dT Primer
- 1 µl RNase inhibitor

Add sterile H₂O to a final volume of 12 µl for each reaction.

Incubate the tubes at 65°C for 5 min.

Cool the tubes on ice for 3 min.

Add the following to each reaction tube:

- 4 µl 5X First-Strand Buffer
- 1 µl DTT (20 mM)
- 2 µl dNTP Mix (10 mM)
- 2 µl SmartRT reverse transcriptase

Incubate the tubes:

50°C for 1.5 hr

85°C for 5 min

Cool down the tubes at 4°C

For regular first-strand cDNA Synthesis

Combine the following in separate 0.2-ml PCR tubes (20 µl reaction):

- 1–10 µl RNA sample
- 1 µl oligo dT Primer or Random Primer
- 1 µl dNTP

Add sterile H₂O to a final volume of 12 µl for each reaction.

Incubate the tubes at 65°C for 5 min.

Cool the tubes on ice for 3 min.

Add the following to each reaction tube:

4 µl 5X First-Strand Buffer

1 µl DTT (20 mM)

2 µl dNTP Mix (10 mM)

2 µl SmartRT Reverse Transcriptase

Incubate the tubes:

50°C for 45 min

85°C for 5 min

Cool down the tubes at 4°C