USB® M-MLV Reverse Transcriptase  
Product number 78306  
Brief protocol  
USB M-MLV Reverse Transcriptase is functionally tested in RT-PCR.  
M-MLV RT and SX M-MLV RT Reaction Buffer may be used in the following protocol to convert mRNA into cDNA:  
Protocol for reverse transcription in RT-PCR  
For 25 µl reaction:  
In first tube, combine RNA and primer:  
10 ng to 1 µg total RNA (or 1 ng to 100 ng polyA RNA)  
10 pmol reverse primer (or 25 pmol oligo dT12-18 [M.W. ~ 4500])  
Add water to 10 µl  
Incubate at 75°C for 5 minutes, then place on ice.  
In second tube, combine reaction components:  
5 µl 5X M-MLV Reaction Buffer  
1.25 µl 10 mM dNTP mix  
100 units M-MLV Reverse Transcriptase  
(Optional: 4 to 20 units RNase Inhibitor)  
Add water to 15 µl  
Combine contents of the two tubes and mix. Incubate at 42°C for 30 minutes, or up to 90 minutes for targets >1.5 kb. Heat inactivate reverse transcriptase at 95°C for 5 minutes. Optionally, treat product with RNase H (PN 70054) to remove RNA template. Use 1 µl product as template per 25 µl PCR reaction.  
Protocol for general reverse transcription:  
For a 50 µl reaction:  
5X M-MLV Reaction Buffer 10.0 µl  
dNTP mix (10 mM each) 2.5 µl  
Oligo (dT)12-18 1 µg  
mRNA 2 - 5 µg  
Ribonuclease Inhibitor (40 units/µl) 1.0 µl  
[α-32P]dNTP (S.A. > 400 Ci/mmol) if required 100 µCi  
M-MLV Reverse Transcriptase (200 units/µl) 2.5 µl  
Water (sterile, DEPC-Treated) __ µl  
Total volume 50 µl  
Incubate at 37°C for 30 minutes. Stop reaction by adding 2 µl of 0.5 M EDTA or by heating to 75°C for 10 minutes. If necessary, the RNA template can be destroyed by adding 10 µl of 5 M NaOH and incubating at 37°C overnight.