

# Super cDNA RT Kit

complete and ready to use kit for cDNA production

**Cat. No:** PR6601

**Storage:** -20°C

**Description:**

Super RT Kit contains M-MLV, optimal reaction buffer, dNTP, RNase inhibitor, RNase-free water and oligo(dT)18. M-MLV is isolated from *E.coli* carrying cloned Moloney murine leukemia virus reverse transcriptase (M-MLV) gene, possesses RNA and DNA dependent polymerase activity and has weak RNase H activity, ensuring high yield of cDNA.

The product can be directly used in 2nd strand synthesis, hybridization, PCR amplification, real-time PCR, etc.

**Components:**

Product	PR6601 (50 reactions)
M-MLV Reverse Transcriptase (200U/μl)	50 μl
5 × First-strand Buffer	250 μl
dNTP Mixture (10 mM each)	50 μl
RNase Inhibitor (40 U/μl)	50 μl
Oligo(dT)18 Primer (50 μM)	50 μl
RNase-free H <sub>2</sub> O	1 ml

**Procedure:**

1. Prepare the reverse transcript reaction solution follow by next:

<u>Total RNA or Poly(A) RNA</u>	<u>0.2-2 μg</u>
<u>Oligo (dT)18 (50 μM)</u>	<u>1 μl</u>
<u>dNTP Mixture (10 mM each)</u>	<u>1 μl</u>
<u>RNase -free dH<sub>2</sub>O</u>	<u>Up to 14 μl</u>

2. Incubate in thermal cycler at 65°C for 5 min, and then fast chill on ice.

3. Add the reverse transcript reaction solution into above PCR tube:

<u>5 × First-strand Buffer</u>	<u>4 μl</u>
<u>M-MLV Reverse Transcriptase (200 U/μl)</u>	<u>1 μl</u>
<u>RNase Inhibitor (40 U/μl)</u>	<u>1 μl</u>
	<u>Total 20 μl</u>

4. Reverse transcription reaction on thermal cycler by next condition:

<u>30°C</u>	<u>10 min</u>
<u>42°C</u>	<u>30-60 min</u>
<u>95°C</u>	<u>5 min</u>

**Note:**

1. The solution used for cDNA synthesis must be treated with DEPC as possible.
2. RNA sample should be avoided genome contamination.
3. Avoid repeated freezing and thawing RNA.
4. Every component should be stored at -20°C.
5. cDNA should be kept at -20°C.

**PCR Procedure:**

1. Transfer 10% volume of the first reaction solution (2 µl) to a new PCR tube.

**Note:** The first reaction solution can be directly used as PCR template without purification, if the dosage is about 1-5 µl. If excessively used, the salt and random primers from the first reaction solution will restrain the activity of DNA polymerase. If purification is needed, it can follow the next: after reaction end of cDNA synthesis (step 6) - add RNase A into reaction system, incubate 10 min at 37°C and use DP1501 to recover cDNA .

2. Add next solution by order:

<u>10 × PCR Buffer</u>	<u>5 µl</u>	
<u>10 mM dNTP mix</u>	<u>1 µl</u>	
<u>10 µM Primer #1 (customer supplied)</u>	<u>1 µl</u>	
<u>10 µM Primer #2 (customer supplied)</u>	<u>1 µl</u>	
<u>H<sub>2</sub>O</u>	<u>x µl</u>	(total reaction volume: 49µl)
<u>Taq DNA polymerase</u>	<u>1 µl</u>	

3. Mix thoroughly and add 50 µl mineral oil to the surface of liquid (optional).
4. Amplification reaction: according to annealing temperature, gene copy number, technical parameters of Taq DNA polymerase, amplification condition, specification of DNA polymerase ,the usual cycle number is 30-35.
5. Detect the product by gel electrophoresis.