



# **FicoScript® M MLV Reverse Transcriptase User Manual**

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FicoScript®Reverse Transcriptase is an engineered version of M-MLV RT with reduced RNase H activity and increased thermal stability. The enzyme is purified to near homogeneity from *E. coli* containing the modified *pol* gene of Moloney Murine Leukemia Virus . The enzyme can be used to synthesize first-strand cDNA at temperatures up to 48°C, providing increased specificity, higher yields of cDNA, and more fulllength product than other reverse transcriptases. It can generate cDNA from 100 bp to >12 kb.It is suitable for qPCR.

- 1 、 Enzyme Storage Buffer:** M-MLV Reverse Transcriptase is supplied in 20mM Tris-HCl (pH 7.5), 200mM NaCl, 0.1mM EDTA, 1mM DTT, 0.01% Nonidet P-40 and 50% glycerol.
- 2 、 M-MLV Reverse Transcriptase 5X Reaction Buffer:** When the M-MLV Reverse Transcriptase 5X Reaction Buffer supplied with this enzyme is diluted 1:5, it has a composition of 50mM Tris-HCl (pH 8.3 25°C ), 75mM KCl, 3mM MgCl<sub>2</sub> and 10mM DTT.
- 3 、 Source:** Purified from an *E. coli* strain expressing a recombinant clone.
- 4 、 Storage Conditions:** Store at – 20°C . Avoid exposure to frequent temperature changes.
- 5 、 Unit Definition:** One unit is defined as the amount of enzyme required to catalyze the transfer of 1nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C . The reaction conditions are: 50mM Tris-HCl (pH 8.3), 7mM MgCl<sub>2</sub> , 40mM KCl, 10mM DTT, 0.1mg/ml BSA, 0.5mM [ 3 H]dTTP, 0.025mM oligo(dT) 50 , 0.25mM poly(A) 400 and 0.01% NP-40.

## 6、 Protocol for First Strand cDNA Synthesis

### 1)、 Reaction Setup

Components	Volume(μl)	Comments
5× RT Buffer	4	Mix thoroughly
random primer	1	Alternatively oligo(dT) or a specific primer can be used.
MLV reverse transcriptase	1	100-200U

RNase inhibitor	1	20-40U
RNA(ul)	X	Max 1µg, usually 2-6ul
DEPC -ddH <sub>2</sub> O (ul)	Y	Add water to fill up to the final reaction volume
Total Volume(ul)	20	

Note: oligo(dT)18 0.5µg or random hexamer 0.2µg or sequence-specific 15-20pmol

## 2)、Cycling Protocol

a)、Protocol using random primer

25°C 10min → 40°C 30 min → 80°C 2 min → 4°C hold

b)、Protocol using oligo(dT)18

25°C 10min → 42°C 30 min → 80°C 2 min → 4°C hold

c)、Protocol using specific gene primer

48°C 30 min → 80°C 2 min → 4°C hold

**7、 Usage Note:** M-MLV Reverse Transcriptase is less processive than AMV Reverse Transcriptase, and therefore, more units of the M-MLV enzyme are required to generate the same amount of cDNA as in the AMV reaction. Thus, starting with 1µg of mRNA in a first-strand cDNA synthesis, 200 units of the M-MLV enzyme are recommended as opposed to 25 units of the AMV enzyme.

- 1). Multiple freeze/thaw of RNA should be avoided. Thaw and keep control RNA on ice.
- 2). It is recommended that the first strand cDNA synthesis is carried out under conditions where RNase contamination has been eliminated. Pipette tips and tubes should be treated with 0.1% diethylpyrocarbonate (DEPC) (soak overnight in 0.1% aqueous solution of DEPC at 37°C, then heat at 100°C for 30 min and autoclave).
- 3). Wearing gloves is highly recommended.

4). Incubation at 40°C will work for most templates, but it can be optimized between 40°C and 48°C if necessary. Raising the temperature can be helpful if the template has strong secondary structures. Higher temperature can also improve specificity if gene-specific primers are used. Incubation time of 30 min is sufficient in most cases. If the target is located near the 5' end of a long transcript and oligo(dT) priming is used, or the target is rare, cDNA synthesis time can be extended up to 60 min.

5). A separate RNA denaturation step is generally not required, but it can be performed before cDNA synthesis if the template RNA has a high degree of secondary structure. The denaturation step, 5 min at 65°C, should be performed before adding 5x RT buffer and reverse transcriptase to the reaction mix.

## 8、References

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