FicoScript® M MLV Reverse Transcriptase User Mannual

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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 2 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 2 , 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(100ng/ul)	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_2O (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℃10min→40℃30 min→80℃2 min→4℃ hold

b)、Protocol using oligo(dT)18

25℃10min→42℃30 min→80℃2 min→4℃ hold

c)、Protocol using specific gene primer

48℃30 min→80℃2 min→4℃ 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℃, then heat at 100℃ 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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