M-MuLV Reverse Transcriptase

Cat # : PT-MMLV Size : $10000U (200U/\mu L)$ SA-MMLV Size : $500U (200U/\mu L)$

Product Description:

Protech M-MuLV Reverse Transcriptase (RT) is a genetically modified M-MuLV RT. It differs from wildtype M-MuLV RT by its structure, catalytic properties and in the optimum activity temperature. The enzyme possesses RNA-dependent and DNA-dependent polymerase activity and a RNase H activity specific to RNA in RNA-DNA hybrids which is significantly lower than that of Avian Myeloblastis Virus (AMV) reverse transcriptase. Protech M-MuLV Reverse Transcriptase activity is optimal at 42°C (active up to 50°C). The enzyme is able to synthesize first strand cDNA up to 13 kb. The enzyme incorporates modified nucleotides.

Components: Store at -20 °C

1. M-MuLV Reverse Transcriptase 50 μ L (SA: 2.5 μ L) The enzyme is supplied in 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 1 mM EDTA, 5 mM DTT, 0.1% (v/v) Triton X-100 and 50% (v/v) glycerol.

2. 5x Reaction Buffer 1 mL (SA: 20 μ L) 250mM Tris-HCl (pH 8.3 at 25°C), 250mM KCl, 20mM MgCl₂, 50mM DTT

General Protocol:

Set up as follows:

1. Mix following component into sterile, nuclease-free tube on ice in the indicated order:

	total RNA	0.1 ng - 5 μg
Template RNA	poly(A) RNA	10 pg – 500 ng
	specific RNA	0.01 pg – 0.5 μg
Primer	Oligo dT	0.05~0.5 μg (10~100 pmol)
	Random hexamer	0.1~0.2 μg (50~100 pmol)
	Gene-specific primer	15-20 pmol
DEPC ddH ₂ O		Total 12.5 µL

2. Incubate at 65 °C for 5 min, chill on ice to open secondary structures of GC rich RNA template. (Optional)

^{**} Please see next page for following information.



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3. Reaction Mix Preparation Table and Reaction Program

Component	Volumn
5x Reaction Buffer	4 μL
RNase Inhibitor	0.5 μL (20U)
dNTP Pre-Mix 10mM	2 μL (1mM final)
M-MuLV Reverse Transcriptase	0.5 μL (100U)*
Template and primer premix	Total 20 µL

^{*} Use 200U per reaction while difficult template problem has been encountered.

Primer Type	Reaction Program
Oligo dT Gene-specific primer	42°C 60min (45°C for GC rich template)
	70°C 10min* (heat-inactivate)
Random hexamer	25°C 10min
	42°C 60min
	(45°C for GC rich template)
	70°C 10min* (heat-inactivate)

 $[\]ensuremath{^{\ast}}$ Do not heat inactivate enzyme prior to analysis of long cDNA to avoid cleavage.

- Metal chelators, inorganic phosphate, pyrophosphate and polyamines are inhibitors of M-MuLV Reverse Transcriptase.
- For experimental sample, please test for your own reaction program.
- The PCR mix is stored in -20 °C for at least 1 year.

Research Use Only

Please do not hesitate to contact us while you have any questions.

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