

Mu-MLV Reverse Transcriptase

Reference: ME-0125-80 • ME-0125-400

Products and procedures described in this protocol are intended for research purposes only.

Applications

Mu-MLV reverse transcriptase is able to synthesize a DNA strand complementary to an RNA template in the presence of a primer. This enzyme is particularly suited for long fragments.

Batch details

Units per vial:	ME-0125-80	8000 units
	ME-0125-400	40000 units
Concentration:	200 U/μl	

Source

The enzyme is isolated from a recombinant *E.coli* clone over expressing the enzyme.

Description

Mu-MLV reverse transcriptase has lower RNase H activity than AMV reverse transcriptase, which is an advantage when synthesizing cDNAs from long mRNAs. Mu-MLV lacks the 3'→5' exonuclease activity. It is recommended to use 10 U/μg RNA for optimum reverse transcription. Although the enzyme shows low activity at 42 °C, it is quite stable at 37 °C.

Package contents

Reagent	Description
Mu-MLV reverse transcriptase	ME-0125-80 ME-0125-400
5x cDNA buffer (blue cap)	250 mM Tris-HCl (pH 8.3), 375 mM KCl, 50 mM DTT, 15 mM MgCl ₂

Shipping conditions

Shipping at 4 °C.

Storage conditions

Storage at -20 °C is recommended.

Storage buffer

50 mM Tris-HCl pH 8.3 (4 °C), 1 mM EDTA, 0.1 % Triton X-100, 0.1 M NaCl, 5 mM DTT, 50 % (v/v) Glycerol.

Analysis conditions

50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 0.5 mM dTTP, 10 mM DTT, 2.5 μM polyA-oligodT 12-18, incubate at 37 °C.

Unit definition

One unit is the amount of enzyme that incorporates 1 nmole of dTTP into acid - insoluble form in 10 minutes at 37 °C using polyA-oligodT 12-18 as substrate.

Reaction procedure

- Thaw all required reagents except for the Mu-MLV reverse transcriptase, which should be kept in the freezer until use. Mix all reagents by inversion and spin them down prior to pipeting.
Note: To correct for dispensing losses prepare an excess of reaction mix. A negative control containing no RNA template should always be included.
- Prepare the RT reaction mix by adding the following components to a nuclease-free 0.2 ml thermocycler tube:
 - x μl oligo d(T)12-18, or random primers, or sequence specific reverse primer

Note: For random primers and oligo d(T)12-18 the final concentration in the reaction mix should be 2.5 μM.

For a sequence-specific reverse primer, the final concentration should be 200 nM.

- 10 ng to 5 μg total RNA or 1 ng to 500 ng mRNA
 - 2 μl dNTP mix 20 mM total (5 mM each dATP, dGTP, dCTP and dTTP)
 - RNase free water to 14 μl
- Mix gently by pipeting.
 - Heat mixture to 65 °C for 5 minutes in a thermocycler and quick chill on ice.
 - Centrifuge briefly to collect the contents to bottom of the tube and add:
 - 4 μl 5x cDNA buffer (blue cap)
 - 2 μl RNase inhibitor at 20 U/μl (Optional but when using less than 100 ng of starting RNA, the addition of RNase inhibitor is essential)
 - Mix contents of the tube gently by pipeting and incubate 3 minutes at 37 °C in a thermocycler.
 - Add 0.5 - 1 μl (100 - 200 units) of Mu-MLV RT (depending on RNA amount)
Note: If less than 1 ng of RNA is used, reduce the amount of Mu-MLV RT in the reaction to 0.25 μl (50 units) and add the RNase free water to 22 μl final volume.
For 10 ng to 1 μg RNA, 0.5 μl (100 units) of Mu-MLV RT can be used.
 - Mix gently by pipeting.
 - Program the thermocycler using the following recommended parameters:

Initial step ¹	10 min at 25 °C
Reverse Transcriptase step	50 min at 37 °C
Inactivation of the RT enzyme	5 min at 95 °C

¹ Only if random primers or oligo d(T)12-18 are used.

The prepared cDNA can now be used as template for amplification in PCR.

Use only 10% of the first-strand reaction (= 2 μl of the RT reaction) for 50 μl PCR reaction. Adding larger amounts of the first-strand reaction may result in decreased amounts of PCR products or may also inhibit the PCR.

Related products

dNTP MIX (Lithium salts)				
Description	Quantity	Volume	Concentration	Reference
dNTP mix	1x 20μmoles	1ml	4x 5 mM	NU-0010-10
	5x 20μmoles	5x 1ml	4x 5 mM	NU-0010-50
	10x 20μmoles	10x 1ml	4x 5 mM	NU-0010-100
dNTP SET (Sodium salts)				
Description	Quantity	Volume	Concentration	Reference
dNTP set	4x 25μmoles	4x 250μl	100 mM each	NU-0060-01
	5x (4x 25μmoles)	5x (4x 250μl)	100 mM each	NU-0060-05
	10x (4x 25μmoles)	10x (4x 250μl)	100 mM each	NU-0060-10

Quality control

➤ RNase Assay

No detectable RNase activity was observed when 10 units of the enzyme was incubated with 8 mg RNA in a 20 μl reaction volume for 24 hours at 37 °C.

➤ Exonuclease Assay

Incubation of 50 units of the enzyme with 1 μg Lambda DNA for 16 hours at 37 °C in the stated reaction buffer does not produce any detectable degradation of the DNA.

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