EUROPE
LIEGE SCIENCE PARK • 4102 Seraing • BELGIUM • Tel.: +32 4 372 74 00 • Fax: +32 4 372 75 00
Toll-free: + 800 666 00 123 • info@eurogentec.com • www.eurogentec.com

NORTH AMERICA
ANASPEC - 34801 Campus Drive • Fremont, CA 94555 • USA • Tel.: +1-510-791-9560
Toll-free: +1 800-452-5530 • Fax: +1 510-791-9572 • service@anaspec.com
www.anaspec.com

Eurogentec
Experience true partnership

Technical **Data Sheet**

Mu-MLV Reverse Transcriptase

ME-0125-400

Eurogentec products are sold for research or laboratory use only and are not to be administrated to humans or used for medical diagnostics.

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-0125-400 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-0125-400
5x cDNA buffer (clear 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 $MgCl_2$, 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
1. 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.

2. 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

EUROPE
LIEGE SCIENCE PARK • 4102 Seraing • BELGIUM • Tel.: +32 4 372 74 00 • Fax: +32 4 372 75 00
Toll-free: 00 800 666 00 123 • info@eurogentec.com • www.eurogentec.com

NORTH AMERICA
ANASPEC - 34801 Campus Drive • Fremont, CA 94555 • USA • Tel.: +1-510-791-9560
Toll-free: +1 800-452-5530 • Fax: +1 510-791-9572 • info.anaspec@eurogentec.com
www.anaspec.com

3. Mix gently by pipeting.

4. Heat mixture to 65 °C for 5 minutes in a thermocyler and quick chill on ice.

5. 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)

6. Mix contents of the tube gently by pipeting and incubate 3 minutes at 37 °C in a thermocycler.

7. 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.

8. Mix gently by pipeting.

9. 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**	**Reference**
dNTP mix	1 x 20 µmoles	1 ml	NU-0010-10
	5 x 20 µmoles	5 x 1 ml	NU-0010-50
	10 x 20 µmoles	10 x 1 ml	NU-0010-100
dNTP SET (Lithium salts)			
Description	**Quantity**	**Volume**	**Reference**
dNTP set	4 x 25 µmoles	4 x 50 µl	NU-0020-10
	5 x (4 x 25 µmoles)	4 x 250 µl	NU-0020-50

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.

For further information please contact our Customer Help Desk:

For Europe:
E-mail: info@eurogentec.com
Tel: +32 4 372 76 65

For USA:
E-mail: info.usa@eurogentec.com
Tel: +1 858 793 26 61