Technical Data Sheet

GENERAL INFORMATION

PRODUCT NAME	Recombinant M-MLV Reverse Transcriptase, lyophilized	
REFERENCE	MT50U-L3MVRTHC	
PRODUCTS PROVIDED	50U-L3MVRTHC	M-MLV Reverse Transcriptase, lyophilized
	RB-L3MVRTHC	1X M-MLV Reverse Transcriptase Reconstitution Buffer
	BMVRTHC	10X M-MLV Reverse Transcriptase Reaction Buffer
	MGCL2	100 mM $MgCl_2$ Buffer
UNITS	50,000 U	
EXPRESSION SYSTEM	*Escherichia coli*	
PURITY	≥ 95 %	

DESCRIPTION

Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) is a RNA-dependent DNA polymerase used to generate first-strand complementary DNA (cDNA) from poly-mRNA, total RNA or viral RNA for use in downstream applications such as RT-(q)PCR or cDNA cloning. This enzyme contains several point mutations in the RNase H domain that enhances its thermostability and increases the cDNA yield of full-length transcripts (>5 kb) than wild-type M-MLV RT.

ADDITIONAL INFORMATION

STORAGE

The lyophilized product can be handled and store at Room Temperature or below until expiry date (see product label). Once reconstituted, it is recommended to store the solution from -20 °C to -80 °C. Avoid multiple freeze/thaw cycles by storing multiple aliquots at -80 °C.

RECONSTITUTION INSTRUCTIONS

1. Spin the vial of the lyophilized enzyme (Ref.: *50U-L3MVRTHC*) at 12,000 x g in a microcentrifuge.
2. Add 250 µL of 1X M-MLV Reverse Transcriptase Reconstitution Buffer (Ref.: *RB-L3MVRTHC*) to reconstitute the enzyme at 200 U/uL.
3. Gently pipette up and down to dissolve the solid powder.
4. Place on ice and aliquot into smaller volumes to avoid multiple freeze/thaw cycles.
5. Store from -20 °C to -80 °C.

Levprot Bioscience, S.L.U.
Polígono Industrial La Charluca, Calle C,
Parcela 10-11
50300 Calatayud, Zaragoza (Spain)
Tel. (+34) 976198404
hello@levprot.com
www.levprot.com

PROTOCOL

1. Gently vortex and briefly centrifuge all solutions.
2. Denature RNA and anneal the primer/s by incubating the RNA with the primer/s at 65-70 °C for 5 min. Then, place the mix on ice for 1 min.

Component	**Final concentration or quantity (recommended)**
RNA Template	Total RNA: up to 5 µg or mRNA: up to 0.5 µg
Primer/s: Oligo dT Random Primers Gene-specific Primers	 1-2 µg Oligo dT 2-5 µg Random Primers ≥ 0.2 µM Gene-specific Primers

3. For reverse transcription of RNA to DNA, complete the mixture from the previous step with the rest of the components in the table to prepare the following 20-µL reaction mix on ice, and mix gently by pipetting:

Component	**Final concentration or quantity (recommended)**
Mix from previous step	
10X M-MLV Reverse Transcriptase Reaction Buffer	1X
dNTPs, 10 mM each	0.5 mM each dNTP
100 mM $MgCl_2$ Buffer	1.5-5 mM
M-MLV Reverse Transcriptase	200 U
Nuclease-free water	Adjust to 20 µL

4. Incubate the mix at 45 °C for 30 min for cDNA synthesis.
5. Inactivate the M-MLV Reverse Transcriptase by incubating the mix at 95 °C for 2 min and then, chill on ice.
6. Proceed with the cDNA in downstream applications or store at -20 °C.

HEALTH AND SAFETY INFORMATION

Consult the Safety Data Sheet for information regarding hazards and safe handling practises. Good Laboratory Practices should be followed when handling this material. The end user assumes all responsibility for care, custody and control of the material, including its disposal, in accordance with the respective national regulations.

QUALITY CONTROL

ANALITICAL SENSITIVITY ASSAY

Analytical sensitivity of each lot of M-MLV Reverse Transcriptase is evaluated performing standard curves in parallel with a reference lot. 10-fold serial dilution of control RNA is performed

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and 5 µL of each dilution are added to 20 µL reaction mixtures containing M-MLV Reverse Transcriptase (100 U), *Taq* DNA polymerase (10 U), specific primers and probe (500 nM and 250 nM respectively), 10X Reaction Buffer (1X), $MgCl_2$ (3 mM) and dNTPs (0.8 mM each). Amplification conditions are those specify for *Taq* DNA Polymerase. Direct detection of PCR products is monitored by measuring the relative fluorescence units (RFU) produced by the result of probe hydrolysis after every cycle. The resulting parallel standard curves are compared and assessed by analyzing the fluorescence, the minimum concentration of nucleic acids detection, the C_t values and the sigmoid shape of the curves.

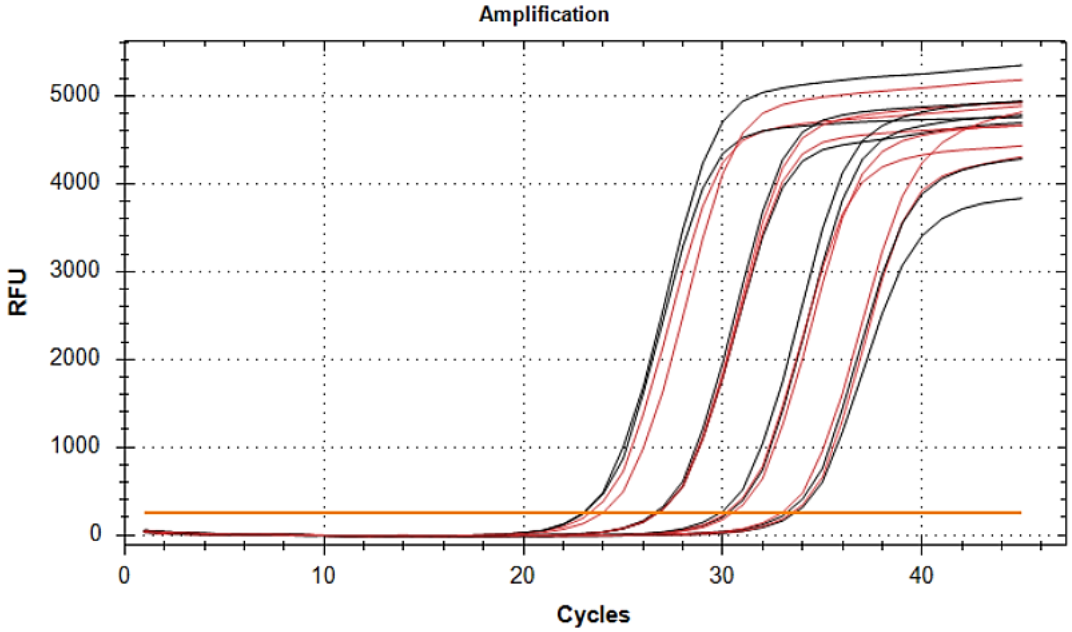

Image: qPCR amplification curves of a reference lot (in black) and an evaluation lot of M-MLV Reverse Transcriptase (in red). Orange line indicated C_t threshold. Similar amplification efficiency is observed.

TECHNICAL SUPPORT

If you have any questions, feel free to contact us at hello@levprot.com

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THIS PRODUCT IS INTENDED FOR RESEARCH USE ONLY.

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VERSION 01