

MMLV Reverse Transcription Kit

Date: 2022.07

Version: 22-01

Cat # : PT-RT-KIT	Size : 100 rxn
SA-RT-KIT	Size : 10 rxn
PT-RT-KIT-10	Size : 1000 rxn
PT-RT-KIT-N	Size : 100 rxn

Product Description:

MMLV Reverse Transcription (RT) Kit is a convenient package of all components which might be used in Reverse Transcription reaction. It's composed of MMLV Reverse Transcriptase, reaction buffer, dNTP, Oligo dT primer, random primer, DEPC water and RNase inhibitor (only available in PT-RT-KIT and SA-RT-KIT). Protech MMLV 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

	Component Name	Volume		Volume		Cap Color
		PT-RT-KIT	SA-RT-KIT	PT-RT-KIT-10	SA-RT-KIT-N	
1	M-MuLV Reverse Transcriptase (200 U/μl)	50 μL	5 μL	50 μL*10	50 μL	White Cap
2	5x Reaction Buffer	1 mL	100 μL	1 mL*10	1 mL	White Cap
3	10mM dNTP	250 μL	25 μL	250 μL*10	250 μL	Blue Cap
4	Oligo (dT) ₁₈ (1 μg/μL)	50μL	5 μL	50μL*10	50μL	Clear Cap
5	Random Hexamers (1 μg/μL)	50μL	5 μL	50μL*10	50μL	Violet Cap
6	DEPC Water	2mL	500 μL	2mL*10	2mL	Orange Cap
7	RNase Inhibitor 50μL (40U/μL)	50μL	5 μL	-	-	Green Cap

General Protocol:

Set up as follows:

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

Template RNA	total RNA	0.1 ng - 5 μg
	poly(A) RNA	10 pg – 500 ng
	specific RNA	0.01 pg – 0.5 μg
Primer	Oligo (dT) ₁₈	0.05-0.5 μg (10-100 pmol)
	Random Hexamers	0.1-0.2 μg (50-100 pmol)

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

3. cDNA Synthesis Mixture Preparation Table and Reaction Program

Component	Volume
5x Reaction Buffer	4 μ L
RNase Inhibitor	0.5 μ L (20U)
dNTP Pre-Mix 10mM	2 μ L (1mM final)
MMLV Reverse Transcriptase	0.5 μ L*
Template and primer premix	From Step 1
DEPC water	x μ L
Total Volume	20 μ L

Primer Type	Reaction Program
Oligo (dT) ₁₈	42°C 60min (45°C for GC rich template)
	70°C 10min* (heat-inactivate)
Random hexamers	25°C 10min
	42°C 60min (45°C for GC rich template)
	70°C 10min (heat-inactivate)

* Use MMLV 1 μ L per reaction while difficult template problem has been encountered.

* MMLV can be purchased separately from Protech (Cat# PT-MMLV)

* RNase inhibitor could be added in a reaction before other components that are a possible source of RNase contamination.

- Metal chelators, inorganic phosphate, pyrophosphate, and polyamines are inhibitors of M-MuLV Reverse Transcriptase.
- For experimental sample, please test for your own reaction program.
- All components of this kit can be stored in -20 °C for at least 1 year.

Product storage & Application:

- cDNA stored at -20°C
- Molecular cloning
- Real-Time PCR
- Microarray analysis

Relative Product:

Product Name	Cat. No	Package
MMLV RT	PT-MMLV	10,000U (200U/ μ L)
dNTP 10mM	PT-8010	1mL
Oligo dT Primer	PT-N420-01	25 μ g
Random Primer	PT-N410-01	25 μ g
UltraPure DEPC Water	PT-P560	1L
RNase Inhibitor	PT-RI01	50 μ L (40U/ μ L)

Form & Storage:

Store at -20°C. This product is stable for 1 year from the date of shipment.

<Summary of Procedure>

Denature:

RNA + Primer
65°C for 5 min

↓
Place on ice for at least 1 min

Anneal:

← Oligo (dT)₁₈

Random Hexamers →

↓
Add cDNA Synthesis Mixture to 20uL (from step3)

↓
25°C for 10 min

cDNA Synthesis:

↙ ↘
42°C for 60 min

45°C for 60 min (GC rich template)

Terminate Reaction:

↓
70°C for 10 min

↓
Application

Research Use Only

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

Manufactured for and distributed by Protech Technology Enterprise Co.,Ltd

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