For research use only

ISO9001

RT Prime kit

Product	Quantity	Cat. No.	Remarks
RT Prime Kit	1 Kit	EBT-1520	Contain all components for reverse transcription

Description

RT Prime Kit is designed for the reverse transcription (RT) from either total RNA or poly A RNA. RT Prime Kit provides reagents sufficient for 50 RT reactions. The kit contains M-MLV (Moloney Murine Leukemia Virus) reverse transcriptase RNase H^-. Both random hexamers and oligo d(T)15 are included, allowing cDNA synthesis from any RNA source. The kit is optimized for 20 μl RT reaction and each cDNA synthesis may be divided and used in up to 20 separate PCR reactions. The kit contains control total RNA and primer set for a positive control experiment.

Kit Component

- M-MLV Reverse Transcriptase RNase H^- : 10,000 unit (200 unit/μl)
- 5x Reverse Transcription Buffer : 500 μl
- 10 mM dNTP : 500 μl
- Random Hexamer : 50 μl (100 pmol/μl)
- Oligo $d(T)_{15}$: 50 μl (100 pmol/μl)
- Recombinant RNase Inhibitor : 2,500 unit (50 unit/μl)
- Total RNA, Human 293 cell : 10 μl (1 μg/μl)
- Control Primer Pair Mix, β-actin : 20 μl (10 pmol/μl)
- RNase-Free Water : 1 ml

Application(s)

First strand cDNA synthesis from total RNA or $polyA^+$ RNA for library construction and RT-PCR.

QC tests

Performance tests.

Storage Condition

Store at -20℃.

㈜ 엘피스바이오텍
www.elpisbio.com
(302-854) 123-12 Chunglim-Dong, Seo-Gu, Taejeon, Korea
Tel: +82-42-581-8448. Fax: +82-42-581-8449

Usage Information (protocol)

1. Add 1 μg total or 100 ng poly A+ RNA sample in a total volume of ≤10㎕ in nuclease-free water.
2. Heat the tube to 70°C for 5 min to melt secondary structure within the RNA template.
3. Cool the tube immediately on ice, then spin briefly to collect the solution at the bottom of the tube.
4. Perform Reverse transcription reaction as follows.

Denatured RNA sample	~ 10 ㎕
5x RT Buffer	4 ㎕
10mM dNTP	1 ㎕
M-MLV RT	1 ㎕
Oligo d(T)15 or Random Hexamer	1 ㎕
Recombinant RNase Inhibitor	1 ㎕(option)
Add RNase-free water to final volume of 20 ㎕	

5. Mix gently by flicking the tube, and incubate for 60 min at 37°C.
6. To stop reaction, incubate for 5 min at 94°C.

Notes

- The cDNA by reverse transcription can be used for subsequent PCR reactions and for the cDNA library constructions.
- If there is concern about possible RNase contamination in the reaction, Recombinant RNase Inhibitor may be added to the reaction to preserve RNA integrity.

PCR

1. Add the following components to the PCR tubes (for 20 ㎕ total reaction).

5x HiPi Premix	4 ㎕
Primers (10 pmol/ ㎕)	0.5 ㎕ each
cDNA by RT reaction	0.1-1 ㎕
Add nuclease-free DW to final volume of 20 ㎕	

2. Perform PCR reaction as follows.

PCR conditions	(100bp – 1kb)		(1-3kb)	
94℃	5 min		5 min	
94℃	30 sec	25-40	30 sec	25-40
50-60℃[a]	30 sec		30 sec	
72℃	45 sec		1.5 min	
72℃[b]	5 min		5 min	

a, Optimal annealing temperature is dependent on the melting point of primer pair
b, Final extension at 72℃ can be omitted if the purpose of PCR is not for a TA cloning
c, The number of PCR cycle is dependent on a copy number of target mRNA.
For a rare copy gene, increase cycle number