For research use only

ISO9001

Reverse Transcription Master Premix (5x)

Product Description	Quantity	Cat. No.	Remarks
Reverse Transcription Master Premix (5x)	0.5 ml	EBT-1511	Contain random hexamer, 5x ready-to-use mix
	0.5 ml	EBT-1512	Contain oligo $d(T)_{15}$, 5x ready-to-use mix
	0.5 ml	EBT-1513	No primer contained, 5x ready-to-use mix

Description

Elpis Biotech's Reverse Transcription Master Premix is a mixture of M-MLV reverse transcriptase (RNase H^+), dNTPs, reaction buffer, and primers (random hexamer (EBT-1511) or oligo $d(T)_{15}$ primer (EBT-1512)) for the first strand cDNA synthesis. Users can perform reverse transcription by simply adding total RNA or poly(A) RNA sample to the premix. Reverse Transcription Master Premixes are supplied as a 5x stock solution. Stabilizer in premix enables prolonging a shelf life at -20℃. If you want to use your own primer pair for cDNA synthesis, you can use the product, EBT-1513, which do not contain any cDNA synthesis primers.

Application(s)

First strand cDNA synthesis from total RNA or $polyA^+$ RNA, Primer extension, RT-PCR.

QC tests

Performance tests.

Storage Condition

Store at -20℃ for one year.

Usage Information (protocol)

1. Add 1μg total or 100 ng $polyA^+$ RNA sample in a total volume of ≤16 μl 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. Add the followings.

Reverse Transcription Master Premix	4 μl
Denatured RNA sample	16 μl

5. Mix gently by flicking the tube, and incubate for 60 min at 37°C or 42°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 μl total reaction).

10x PCR Buffer	2 μl
dNTP mix (2.5 mM each)	1.6 μl
Primers (10 pmol/μl)	0.5 μl each
cDNA by RT reaction	0.1-1 μl
Taq (5 unit/μl)	0.2 μl
Add nuclease-free DW to final volume of 20 μl	

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

㈜ 엘피스바이오텍
www.elpisbio.com
(302-152) Maebong B/D 2F, 23-6 Doma-dong, Seo-Gu, Taejeon, Korea Tel: 042-527-5681-2. Fax: 042-527-5683