



For research use only

ISO9001

## Reverse Transcription Premix (96 Tubes, 5x)

Product	Qty.	Cat. No.	Remarks
Reverse Transcription	96 tubes	EBT-1514	Contain random hexamer, 5x ready-to-use mix, aliquoted
Master Premix	96 tubes	EBT-1515	Contain oligo d(T) <sub>15</sub> , 5x ready-to-use mix, aliquoted
(5x, 20 µl reaction)	96 tubes	EBT-1516	No primer contained, 5x ready-to-use mix, aliquoted

### Description

Elpis Biotech's Reverse Transcription Premix is a mixture of M-MLV reverse transcriptase (RNase H<sup>+</sup>), dNTPs, reaction buffer, and primers (random hexamer (EBT-1514) or oligo d(T)<sub>15</sub> primer (EBT-1515)) for the first strand cDNA synthesis. Reverse Transcription Premix is pre-aliquoted to 4 µl as a 5 x concentrated form into individual PCR tubes in order to perform 20 µl RT reactions. Users can perform reverse transcription by simply adding total RNA or poly(A) RNA sample to the premix. Stabilizer in the premix enables prolonging a shelf life at -20°C. If you want to use your own primer pair for cDNA synthesis, you can use the product, EBT-1516, which do not contain any cDNA synthesis primers.

### Application(s)

First strand cDNA synthesis from total RNA or polyA<sup>+</sup> RNA, Primer extension, RT-PCR.

### QC tests

Performance tests.

### Storage Condition

Store at -20°C for one year.

### Usage Information (protocol)

1. Add 1 µg total or 100 ng polyA<sup>+</sup> RNA sample in a total volume of ≤17 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 16 µl denatured RNA sample to the tubes containing Reverse Transcription Premix (4 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

- If you intend to use your own primers for the first stand cDNA synthesis (when using EBT-1516), add primers at step 1.
- 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	(100 bp – 1 kbp)	(1-3 kbp)
94°C	5 min	5 min
94°C	30 sec	30 sec
50-60°C <sup>a</sup>	30 sec	30 sec
72°C	45 sec	1.5 min
72°C <sup>b</sup>	5 min	5 min

a. Optimal annealing temperature is dependent on the melting point of primer pair  
b. Final extension at 72°C 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