For research use only

ISO9001

# HiPi RT-PCR Kit

| Product | Quantity | Cat. No. | Remarks |
|---|---|---|---|
| **HiPi RT-PCR Kit** | 1 Kit | EBT-1103 | Contain all components for RT-PCR |

## Description

HiPi RT-PCR Kit is designed for the reverse transcription (RT) and polymerase chain reaction (PCR) for detection of specific genes from either total RNA or poly A RNA. HiPi RT-PCR Kit provides reagents sufficient for 50 RT reactions and 500 PCR reactions.

The kit contains M-MLV (Moloney Murine Leukemia Virus) reverse transcriptase RNase $H^+$ and HiPi Plus thermostable DNA polymerase. 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 total RNA and primer set for a positive control experiment.

## Kit Component

- M-MLV Reverse Transcriptase : 10,000 unit (200 unit/μl)
- 5x Reverse Transcription Buffer : 500 μl
- HiPi Plus Thermostable DNA Polymerase : 500 unit (5 unit/μl)
- 10x HiPi plus Reaction Buffer : 1 ml
- 10 mM dNTP : 1 ml
- Random Hexamer : 50 μl (100 pmol/μl)
- Oligo $d(T)_{15}$ : 50 μl (100 pmol/μl)
- Recombinant RNase Inhibitor : 2500 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 : 1ml

## Application(s)

First strand cDNA synthesis from total RNA or $polyA^+$ RNA, and subsequent PCR reaction.

## 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

### First Strand cDNA Synthesis

1. Add 1 μl of 100 pmol/μl primer (random hexamer or oligo $d(T)_{15}$) or 10 pmol/μl gene specific anti-sense primer per microgram of the total RNA sample in a total volume of ≤10 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 to the primer/RNA mix in the order shown.

| | |
|---|---|
| M-MLV 5X Reaction Buffer | 4 μl |
| dNTP mix (2.5 mM each) | 4 μl |
| M-MLV RT 200 units | 1 μl |
| Add nuclease-free DW to final volume of 20 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 HiPi plus Reaction 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 |
| HiPi plus 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