Yeastern Biotech Co., Ltd

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This product is intended to be used for life science research only. It has not been approved for drug or diagnostic purpose. YEASTERN's products should not be resold, modified for resale, or used to manufacture commercial products without written approval by YEASTERN. YEASTERN guarantees the performance of all products in the manner described in our protocol. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, YEASTERN will replace it free of charge.

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# Deoxy+ RT Kit

**Cat. No.**
FYT531-100P

# Deoxy+ RT Kit

**Storage:** - 20 ℃

## Description

Deoxy+ RT Kit is composed of Deoxy+ HiSpec Reverse Transcriptase (RTase), 2X Deoxy+ RT premix, RNase inhibitor, and primers. Deoxy+ HiSpec RTase is genetically engineered by introducing point mutations to MMLV RTase that increase half-life, reduce RNase activity and increase thermal stability. Those mutations also lead to increased specificity of Deoxy+ HiSpec RTase and giving highest cDNA yields. It is ideal for RT-PCR of a specific target gene or generating cDNA from total or poly (A)+ RNA samples. It synthesizes a complementary DNA strand from total RNA, mRNA, or an RNA:DNA hybrid. This kit also includes RNase inhibitor, which specifically inhibits RNases A, B and C with high affinity, thus protects template RNA from degradation by RNase during the reverse transcription reaction.

## Content

- Deoxy+ HiSpec Reverse Transcriptase
- 2✕ Deoxy+ RT premix : 100 mM Tris-HCl pH 8.3，150 mM KCl，6 mM $MgCl_2$，20 mM DTT，1 mM dNTPs
- RNase Inhibitor (40 U/μL)
- 50 μM Oligo (dT)
- 50 μM Random Primer

## Unit Definition

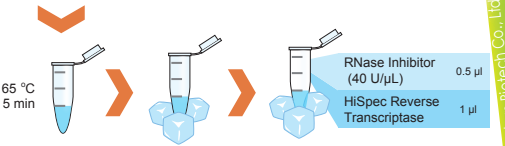
One unit incorporates 1 nmole of dTTP into acid precipitable material in 10 min at 37 °C using poly(A)-oligo(dT) as template primer.

## Standard Protocol for First-Strand cDNA Synthesis

Add the following components to the microtubes on ice.

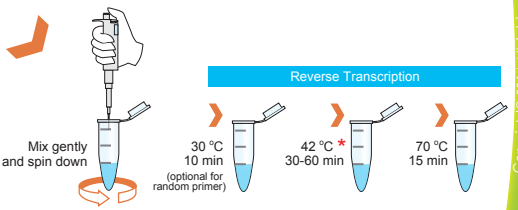
| | |
|---|---|
| 50 μM Oligo (dT) primer | 1 μl |
| or 50 μM Random primer | 1 μl |
| or Gene specific primer | 2 pmole |
| 2✕ Deoxy+ RT premix | 10 μl |
| Template RNA (total RNA ≤ 5μg or mRNA ≤ 1μg) recommended : 100~500 ng | |
| Nuclease-Free Water | variable |

Total volume 18.5 μl




Cool immediately on ice for 30 sec and spin down

Add the following components to the microtubes.




* If needed, the RT reaction temperature can be increased to 50°C to help process RNA with secondary structures.

## PCR (Recommended)

Use only 2 μl of the first-strand reaction for PCR.

1. Add the following components to a PCR tube.
   - 10✕ PCR Buffer ..... 5 μl
   - 10 mM dNTPs Mixture ..... 1 μl
   - 10 μM Forward primer ..... 1 μl
   - 10 μM Reverse primer ..... 1 μl
   - 5 U/μl Taq DNA polymerase ..... 1 μl
   - The first-strand reactant ..... 2 μl
   - $ddH_2O$ ..... to 50 μl
2. Mix gently and spin down.
3. Perform 20 to 40 cycles of PCR.