



Yeastern Biotech Co., Ltd

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**Address:** 6F-3, 23 Lane 169, Kang Ning St.,  
Shijr, Taipei, 22180 Taiwan.

**Tel:** +886-2-2695-3922 **Fax:** +886-2-2695-3979

**Email:** yeastern@yeastern.com.tw



# Deoxy<sup>+</sup>



## RT Kit

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

# Deoxy<sup>+</sup> RT Kit

Storage: - 20 °C

## Description

Deoxy<sup>+</sup> RT Kit is composed of Deoxy<sup>+</sup> HiSpec Reverse Transcriptase (RTase), 2X Deoxy<sup>+</sup> RT premix, RNase inhibitor, and primers. Deoxy<sup>+</sup> 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<sup>+</sup> 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)<sup>+</sup> 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<sup>+</sup> HiSpec Reverse Transcriptase
- 2X Deoxy<sup>+</sup> RT premix : 100 mM Tris-HCl pH 8.3 , 150 mM KCl , 6 mM MgCl<sub>2</sub> , 20 mM DTT , 1 mM dNTPs
- RNase Inhibitor (40 U/μL)
- 50 μM Oligo (dT)
- 50 μM Random Primer

## Unit Definition

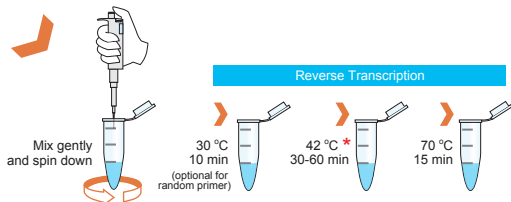
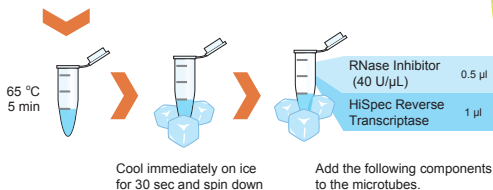
One unit incorporates 1 nmole of dTTP into acid precipitable material in 10 min at 37 °C using poly(A)-oligo(dT) as template primer.

## 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   |
| ● 2X Deoxy <sup>+</sup> RT premix | 10 μl   |
| ● Template RNA                    | (total RNA ≤ 5μg or mRNA ≤ 1μg)<br>recommended : 100–500 ng |
| ● Nuclease-Free Water             | variable  |

Total volume 18.5 μl



\* 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.

10X 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 <sub>2</sub> O	to 50 μl
2. Mix gently and spin down.
3. Perform 20 to 40 cycles of PCR.