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Ver. L0307

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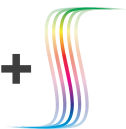
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Deoxy⁺



HiSpec Reverse Transcriptase

Cat. No.

FYT501-50R

FYT501-100R

Deoxy⁺ HiSpec Reverse Transcriptase

Concentration: 200 U/μl

Storage: - 20 °C

Description

Deoxy⁺ HiSpec Reverse Transcriptase (RT) is genetically engineered by introducing point mutations to MMLV RT that increase half-life, reduce RNase activity and increase thermal stability. Those designed mutations lead to increased specificity of Deoxy⁺ HiSpec RT and the highest cDNA yield of all RTs. It is ideal for RT-PCR of a specific 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.

Content

- Deoxy⁺ HiSpec Reverse Transcriptase
- 2× Deoxy⁺ RT premix:
100 mM Tris-HCl pH 8.3 , 150 mM KCl , 6 mM MgCl₂ , 20 mM DTT ,
1 mM dNTPs

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.

- | | |
|--|----------|
| ● Oligo (dT) primer | 50 pmole |
| or Random primer | 50 pmole |
| or Gene specific primer | 2 pmole |
| ● 2× Deoxy ⁺ RT premix | 10 μl |
| ● Template RNA (total RNA ≤ 5μg or mRNA ≤ 1μg) | |
| ● ddH ₂ O | variable |

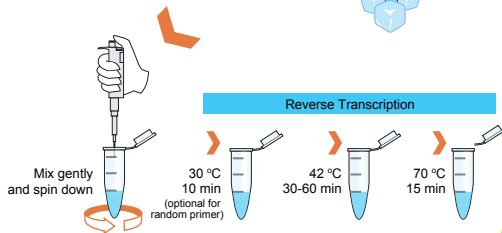
Total volume 18 μl

65 °C
5 min

Cool immediately on ice for 30 sec and spin down

Add the following components to the microtubes.

- | | |
|------------------------------|------|
| RNase Inhibitor (optional) | 1 μl |
| HiSpec Reverse Transcriptase | 1 μl |



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 ₂ O	to 50 μl

2. Mix gently and spin down.
3. Perform 20 to 40 cycles of PCR.