

TransScript® II Two-Step RT-PCR SuperMix

Cat. No. AH401

Storage: at -20°C for one year

Description

TransScript® II Two-Step RT-PCR SuperMix performs first-strand cDNA synthesis and PCR in two steps. 5×TransScript® II All-in-One SuperMix for PCR is used for reverse transcription and 2×TransTaq® HiFi PCR SuperMix II is used for PCR.

Highlights

Amplification of fragment up to 15 kb.

Applications

- cDNA library construction, 3' and 5' RACE
- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template

Kit Contents

| Component | AH401-01 |
|---|----------|
| 5×TransScript® II All-in-One SuperMix for PCR | 200 µl |
| 2×TransTaq® HiFi PCR SuperMix II | 2×1 ml |
| RNase-free Water | 1 ml |

Procedures

First-strand cDNA synthesis

1. Reaction Components

| Component | Volume |
|---|---------------------|
| Total RNA/mRNA | 50 ng-5 µg/5-500 ng |
| 5×TransScript® II All-in-One SuperMix for PCR | 4 µl |
| RNase-free Water | to 20 µl |

Optional: for higher efficiency, suggest to mix RNA, primer and water first. Incubate the mixture at 65°C for 5 minutes, on ice for 2 minutes. Then add other components.

2. Incubation

- For RNA template with poly(A)⁺, incubate at 50°C for 30 minutes.
- For RNA template without poly(A)⁺, incubate at 25°C for 10 minutes, then at 50°C for 30 minutes.
- For GC-rich or complex secondary structure RNA template, incubate at 55°C for 30 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

Reaction Components

| Component | Volume | Final Concentration |
|--|------------|---------------------|
| Template | Variable | as required |
| Forward Primer (10 μ M) | 1 μ l | 0.2 μ M |
| Reverse Primer (10 μ M) | 1 μ l | 0.2 μ M |
| 2 \times <i>TransTaq</i> [®] HiFi PCR SuperMix II | 25 μ l | 1 \times |
| ddH ₂ O | Variable | - |
| Total volume | 50 μ l | - |

Thermal cycling conditions

| | | |
|---------|------------|----------------|
| 94°C | 2-5 min | } 35-40 cycles |
| 94°C | 30 sec | |
| 50-60°C | 30 sec | |
| 72°C | 1-2 kb/min | |
| 72°C | 5-10 min | |

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