TransScript® Fly First-Strand cDNA Synthesis SuperMix

Cat. No.: AF301

Storage: at -20°C for two years

Description

TransScript® Fly RT is generated by site mutations. It provides high affinity to RNA template with fast extension rate. The cDNA is efficiently synthesized by *TransScript*® Fly RT/RI Enzyme Mix and 2×TS Fly Reaction Mix. The entire reverse transcription can be completed within 5 minutes.

- 5 minutes reverse transcription.
- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- Anchored Oligo(dT)$_{18}$ Primer for higher yield and more full length cDNA.
- cDNA up to 12 kb.

Application

Multiple copy and low copy gene fast detection

Kit Contents

Component	AF301-02	AF301-03
TransScript® Fly RT/RI Enzyme Mix	50 µl	100 µl
2×TS Fly Reaction Mix	500 µl	1 ml
Random Primer(N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

First-Strand cDNA Synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng -5 µg/5-500 ng
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg /µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×TS Fly Reaction Mix	10 µl
TransScript® Fly RT/RI Enzyme Mix	1 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, ice for 2 minutes. Then add other components.

2. Incubation

- For anchored oligo(dT)$_{18}$ primer or GSP, incubate at 42°C for 5 minutes.
- For random primer, incubate at 25°C for 10 minutes, then at 42°C for 5 minutes.

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

RT-PCR

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2×*TransTaq*® HiFi PCR SuperMix II	25 μl	1×
Nuclease-free Water	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

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

Notes

• Avoid RNase contamination.

• 5 minutes RT at 42°C is sufficient for most cDNA synthesis. For some genes, longer (upto 15 minutes) reaction time may needed.

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