

FastGene® Scriptase III cDNA Kit

Cat. No.	Pack Size
LS66	100 reactions
LS66s	10 reactions

Storage:

Store at -20°C.

Description:

FastGene® Scriptase III is an improved version of M-MLV Reverse Transcriptase, which exhibits high thermal stability (~55°C) and low RNase H activity, and can synthesize cDNA up to 12 kb in length.

Characteristics:

- Produces excellent yields of amplifiable cDNA.
- Can synthesize long cDNA targets, up to 12 kb, from small amounts of input RNA.
- Able to reverse transcribe through RNA secondary structures.
- Strict enzyme purity specifications.

Applications:

- cDNA cloning or cDNA library preparation.
- Two step RT-PCR (end-point or real-time quantitative PCR).

Quality control:

- Purity: >99% on SDS-PAGE
- Endonuclease- and exonuclease-free
- RNase-free
- Inhibitor-free
- Satisfactory yield and length of cDNA products

Protocol:

1. Prepare the PCR mixture on ice with the following **components included in this kit**:

10× RT Buffer	2 µl
FastGene® Scriptase III (200 units/µl)	1 µl
dNTP Mixture (10 mM each)	1 µl
0.1 M DTT	2 µl
Template RNA* (not included)	x µl
Primer**	1 µl
RNase Inhibitor (40 units/µl)	0.5 µl
25 mM MgCl ₂	4 µl
Sterile water (RNase free)	up to 20 µl

* Concentration of template RNA

Total RNA: 1 pg~5 µg

Messenger RNA (mRNA): 1 pg~500 ng

** Prepare one of the following primers

Oligo (dT)18: 50-100 µM

Random hexamer: 50-100 µM

Specific primer: 15-20 pmol

An additional annealing step is recommended if using random hexamer. Incubate at 25° for 10 min.

2. Incubate at 50° for 50 min.

3. Incubate at 85° for 5 min to inactivate the reaction.

Note:

FastGene® Scriptase III excels at 50°C but, can be adjusted within the range of 42°-55°C based on experimental needs. RNAs containing secondary structure and other challenging targets can be synthesized at 55°C without compromising performance. The incubation time for the synthesis can be decreased to a minimum of 30 min.

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