

FastGene[®] Scriptase II

cDNA Synthesis Kit

Manual

LS63

Version 1.2

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Store at -20°C

 **Genetics** NIPPON Genetics EUROPE

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Product description

Reverse transcription

The ability to reverse the process of translation been a crucial element of modern molecular biology. The enzyme, a RNA-dependent DNA-Polymerase is called reverse transcriptase. Reversing the messenger RNA delivers the complementary DNA, which can then be detected or quantified using PCR and qPCR assays.

Due to the lack of the 3'→5' exonuclease activity, the MuLV has become more and more popular. The MuLV has also been used as a template to engineer new enzymes with additional features – reduced RNase H activity or higher processivity. The FastGene Scriptase II is a modified MuLV RT with a reduced RNase H activity.

Kit Content (100 Reactions)

- FastGene®Scriptase II (200 U/μl)
- 5x FastGene® RT buffer
- DTT (0.1 M)
- dNTP Mixture (2 mM each)
- Sterile water (RNase free)
- RNase Inhibitor (40 U/μl)
- Oligo dT Primer (80 μM)
- Random hexamer (100 μM)

Quality Control

- Purity: >99% on SDS-PAGE
- Endonuclease-free
- Exonuclease-free
- RNase-free
- Inhibitor-free
- Satisfactory Yield

Concentration of template RNA

- *Total RNA: 1 ng-5 µg*
- *Messenger RNA (mRNA): 1 ng-0.25 µg*
- *Specific RNA: 0.01 pg-0.5 µg*

1. Mix the recommended concentration of RNA template with 1 µl oligo dT primer and/or 1 µl random hexamer or 1 µl of gene specific primer
2. Add 2 µl of dNTP
3. Add distilled Water (sterile) to obtain 12.5 µl
4. Heat the mixture to 65 °C for 5 min → quickly chill on ice.
5. Add the following components:

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|----------------------------------|--------|
| 5x FastGene® Scriptase II buffer | 4 µl |
| 0.1 M DTT | 2 µl |
| RNase Inhibitor | 0.5 µl |

6. Incubate at 42 °C for 2 minutes.

Note: Change the additional annealing step to **2 minutes at 25°C** if random hexamers are used.

7. Store the RNA mixture on ice and add 1 µl of FastGene® Scriptase II
8. Incubate at 42°C for 50 min.

Note: FastGene® Scriptase II performs optionally over the full range of 42°C-50°C.

9. Incubate 70 °C for 15 min for complete enzyme deactivation.

FastGene[®] Scriptase II

cDNA Synthesis Kit

Please contact us for additional information:

info@nippongenetics.eu

Please contact our technical support:

support@nippongenetics.eu

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|------|---|---------------|
| LS52 | FastGene[®] Scriptase Basic (20.000 units at 200 U/ μ l) | 100 Reactions |
| LS62 | FastGene[®] Scriptase Basic cDNA Synthesis containing Oligo dTs, random hexamer and RNase inhibitor | 100 Reactions |
| LS53 | FastGene[®] Scriptase II (20.000 units at 200 U/ μ l) | 100 Reactions |
| LS63 | FastGene[®] Scriptase II cDNA Synthesis containing Oligo dTs, random hexamer and RNase inhibitor | 100 Reactions |

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