FaithScriptor G4th RT Supermix

Cat. No.: 2209-100 Size: 100 rxns

Storage Condition

Store at -20℃

Components

Component	Size
FaithScriptor G4th RT Supermix	400 µl
dsDNase	2×50 µl
10× dsDNase Buffer	200 µl
Nuclease-Free Water	2×1 ml

Description

FaithScriptor G4th RT Supermix (with dsDNase) is a simplified system specially designed for single-stranded cDNA synthesis. It contains all components required for the reaction, requiring only the addition of RNA template and water.

The FaithScriptor G4th Reverse Transcriptase in the premix is a newly developed 4th-generation reverse transcriptase. It features faster reaction speed, completing the reverse transcription process within 5 minutes; stronger thermostability with an optimal reaction temperature of 55 °C; the cDNA product can reach up to 20 kb in length. Additionally, it exhibits tolerance to common inhibitors such as ethanol, isopropanol, humic acid, lithium chloride, and guanidine hydrochloride.

dsDNase can specifically digest double-stranded DNA even in the presence of primers and probes, without degrading single-stranded DNA or RNA. It exhibits thermal sensitivity, enabling rapid irreversible inactivation at elevated temperatures to eliminate genomic contamination. This prevents dsDNase from damaging DNA within DNA-RNA hybrid strands during reverse transcription.

Protocol

For RNA samples with high genomic DNA content

1. Genomic DNA contamination removal

① Prepare the following reaction mixture on ice:

Reagent	Amount
Template RNA[a]	50 ng~1 µg
dsDNase	1 µl
10× dsDNase Buffer	1 µl
Nuclease-Free Water	To 10 µl

a. It is recommended to use RNA extracted from a kit as a template.

② Mix gently and spin down.

③ Incubate at 37℃ for 2 minutes to remove genomic DNA contamination.

Note: If the RNA has severe contamination of genomic DNA, the incubation time at 37℃ can be appropriately extended to 5 minutes.

④ Incubate the mixture at 65℃ for 2 minutes to inactivate dsDNase. Place the mixture on ice afterward.

2. First-strand cDNA synthesis

① Prepare the following reaction mixture on ice:

Reagent	Amount
Product from previous reaction	10 µl
FaithScriptor G4th RT Supermix	4 µl
Nuclease-Free Water	To 20 µl

② Mix gently and spin down.

③ Incubate at 55℃ for 5 minutes.

Note: If the template RNA does not contain a poly(A) tail, you may pre-incubate at 25℃ for 10 minutes.

④ Terminate the reaction by incubating at 85℃ for 5 minutes.

⑤ Quickly place the obtained cDNA on ice for subsequent experiments.

Note: cDNA solution can be stored at -20 ℃ for up to 1 week, long-term storage is recommended at -80℃ .

For RNA samples with low genomic DNA content

① Prepare the following reaction mixture on ice:

Reagent	Amount
Template RNA[a]	50 ng~1 µg
FaithScriptor G4th RT Supermix	4 µl
dsDNase	1 µl
Nuclease-Free Water	To 20 µl

a. It is recommended to use RNA extracted from a kit as a template.

② Mix gently and spin down.

③ Incubate at 37℃ for 2 minutes to remove genomic DNA contamination.

④ Incubate at 55℃ for 5 minutes.

⑤ Terminate the reaction by incubating at 85℃ for 5 minutes.

⑥ Place the obtained cDNA solution on ice for subsequent experiments.

Note: The cDNA solution can be stored at 20℃ for up to one week. Long-term storage is recommended at 80℃ .

Notice

The premix contains Oligo(dT)$_{20}$VN and random primers, suitable not only for eukaryotic mRNA containing poly(A) tail, but also for templates that do not contain poly(A) tail, such as prokaryotic RNA, eukaryotic rRNA, tRNA, etc. However, it is not suitable for small RNA like miRNA.

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