GIII Visual All-in-One MasterMix (with dsDNase)

REF: EG26102S

Storage Condition

Store at -20℃ for 2 years.

Components

Component	Amount
GIII Visual All-in-One MasterMix	400 μl
dsDNase	2×50 μl
10× dsDNase Buffer	200 μl
Nuclease-Free Water	2×1 ml

Description

GIII Visual All-in-One MasterMix (with dsDNase) is a high-quality, efficient, and convenient one-step cDNA synthesis kit. It is designed to minimize contamination and contains all the necessary components for first-strand cDNA synthesis, including thermostable M-MLV GIII Reverse Transcriptase and its reaction buffer, RNase inhibitor, dNTPs, Oligo$(dT)_{20}$VN, and random primers-all the necessary components. Simply add RNA template and water to initiate the reaction. Compared to conventional reverse transcription mixes, this product eliminate pipetting errors with non-interfering, visible tracking dye. The cDNA obtained using this kit is primarily used for downstream qPCR experiments.

RNA extracted from tissues and cells often contains genomic DNA contamination. If it is not removed prior to reverse transcription, both genomic DNA and cDNA can be amplified during subsequent qPCR reactions (especially when the primers are designed within the same exon), which can affect the accuracy of gene expression quantification. This kit employs dsDNase to efficiently remove genomic DNA contamination. DsDNase specifically digests double-stranded DNA (dsDNA) or DNA strand in DNA-RNA hybrid chains. It is heat-labile and can be rapidly and irreversibly inactivated at the reverse transcription temperature. Compared to traditional methods that use DNase I to remove genomic DNA contamination, dsDNase does not require the addition of EDTA for inactivation. This not only saves experimental time but also reduces the inhibition of reverse transcription.

Two protocols can be choosen based on the content of genomic DNA contamination: two steps that separate genomic DNA removal and reverse transcription, or one-step method that combines genomic DNA removal and reverse transcription.

Protocol

1. For RNA samples with low genomic DNA content (Recommended Protocol)

① Prepare the following reaction mixture on ice:

Reagent	Amount
Template RNA[a]	50 ng~1 μg
GIII Visual All-in-One MasterMix	4 μl
dsDNase	1 μl
Nuclease-Free Water	To 20 μl

a. It is recommended to use high-quality RNA extracted with kit as the template.

② Mix gently and spin down.

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

④ Incubate at 55℃ for 15 minutes.

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

⑥ Quickly place the cDNA product on ice for subsequent experiments, or immediately store it at -20℃ .

2. 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 with kit as the template.

② Mix gently and spin down.

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

Note: If the genomic DNA contamination is severe in the RNA sample, the incubation time at 37℃ may be 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 (Experimental Group)
"Experiment (1)" product	10 μl
GIII Visual All-in-One MasterMix	4 μl
Nuclease-Free Water	To 20 μl

② Mix gently and spin down.

③ Incubate the mixture at 50℃ for 15 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 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℃ .

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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.

Since random primers can initiate reverse transcription at any position on the RNA, it is not recommended to use this product for full-length cDNA cloning of eukaryotic organisms. For obtaining full-length cDNA of eukaryotic organisms, we recommend RTase III Primer Flexible All-in-One Mix (with dsDNase) (EG24102).

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