MIIIs™ 1st Strand gDNA clear cDNA Synthesis SuperMix

Cat. No : MIII-0050 Size : 50 rxn/kit Storage : -20℃

Description

MIIIs™ 1st Strand gDNA clear cDNA Synthesis SuperMix is a ready-to-use master mix for removing genomic DNA(gDNA) and first-strand cDNA synthesis. It contains MIIIs™ Reverse Transcriptase, DNase I, dNTPs, OligodT and Random Primer mix and an optimized buffer system. Only add template RNA and ddH_2O can be reacted.

Kit Components

gDNA clear / 50 µl 、10X Reaction Buffer / 50 µl、Stop Buffer /50 µl 、ddH_2O /1000 µl

Reverse Transcriptase MIIIs / 50 µl 、5X First-Strand Buffer / 250 µl 、Oligo dT&Random primer / 100 µl

1. Residual genomic DNA removal

Component	Volume
10X Reaction Buffer	1 µl
gDNA clear	1 µl
Template RNA (total RNA 10ng ≦ 2 µg or mRNA 10ng ≦ 1µg)	- µl
RNase-Free Water	up to 10µl
Heat at 37℃ for 5 mins 、 On ice 1 mins	
Stop Buffer	1 µl
65℃ for 5 mins to denature DNase I	
total gDNA clear sample	11 µl

2. Standard protocol for First-Strand

Component	Volume
gDNA clear sample	11 µl
Oligo dT&Random primer	2 µl
5X First-Strand Buffer	4 µl
Reverse Transcriptase MIIIs 200U/µl	1 µl
RNase-Free Water	up to 20µl
Mix gently and spin down	
Heat at 48℃ for 15 mins **Heat at 85℃ for 5 mins to denature RTase.**	
Store products at-20℃ proceed to PCR using 2µl first-strand cDNA synthesis reaction mixture.	

PCR amplification

Use only 2µl of the First-Strand reaction for PCR **Add the following components to a PCR tube**	
Component	Volume
10X PCR Buffer	5 µl
10 mM dNTPs mix	1 µl
10µM Forward primer	1 µl
10µM Reverse primer	1 µl
5U/µl Taq DNA polymerase	0.5 µl
The First-Strand reactant	2 µl
Autoclaved , distilled water	to 50 µl
Mix gently and spin down . Perform 30 to 40 cycles of PCR .	

qPCR

Use only 2µl of the First-Strand reaction for PCR	
2X qPCR Premix	12.5 µl
10µM Forward primer	0.75 µl
10µM Reverse primer	0.75 µl
The First-Strand reactant	2 µl
Autoclaved , distilled water to 25µl • Mix gently and spin down . • perform 30 to 40 cycles of qPCR .	

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