Differentiating Research

Product Details

Phi-Script™ 1st Strand cDNA Mastermix(5X)

Product Overview

Phi (ϕ) Script ™ 1st strand cDNA Mastermix is extremely user friendly while making cDNA. The master-mix (5X) is uniquely blended with RNase Inhibitors, dNTPs, Mg+2, Reaction buffers, oligo(dT) , random primers and Stabilisers. Our flagship enzyme PhiScript reverse transcriptase is provided in a separate vial. t's an engineered form of Moloney Murine Lukemia Virus reverse transcriptase (M-MuLV-RT). Point mutations are introduced in ϕScript™ reverse transcriptase so as to reduce RNAse H Activity and to improve its thermal stability.

Catalog Details

R6203 20µLX50 Reactions

R6204 20µLX250 Reactions

Single Pack Contains

- cDNA Mastermix (5X),200µL
- PhiScript Reverse Transcriptase (200U/µL), 50µL
- Nuclease Free Water

CRITICAL NOTE

≤ 2KB

This mastermix is generally optimized for target amplicon size ((≤2kb). However, longer transcripts can also be achieved by changing incubation time.

First Strand cDNA Synthesis Protocol

The most important element is - good quality RNA. Make sure you have extracted quality RNA (Total RNA or mRNA) from appropriate cells . The quality of the RNA can be checked using agarose gel electrophoresis to see two distinct rRNA. Make sure to have all the tips, plastic wares, other consumables and working area RNAse Free. THIS MASTERMIX IS NOT COMPATIBLE FOR GENE SPECIFIC cDNA SYNTHESIS OR ONE-STEP RT PCR ASSAYS.

Components	20µL Reaction	Target
RNA Template	upto 15µL	Upto 1µg (More than this would inhibit the reaction
DX/DT cDNA Mastermix (5X)	4 µL	1X
PhiScript™ Reverse Transcriptase (200U/µL)	1 µL	200 U - 400U
Nuclease Free Water	Make upto 20µL	
Total Volume	20 µL	

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cDNA Synthesis Method

Step 1: Incubate at 25℃ for 5 minutes

Step 2: Incubate 42℃ for 20 **minutes** (For targets ≤ 2KB)

OR

Incubate 42℃ for **30-60** minutes (For Targets ≥ 2KB)

Step 3: Incubate at 95℃ for 2 minutes. This is to inactivate Reverse Transcriptase.

Step 4: Store the cDNA Sample at -20 ℃ for further downstream analysis.

Notes:

- For complex RNAs you can change the Reverse Transcription temperature to upto 60**℃**.
- DX/DT PhiScript™ cDNA Mastermix (5X) contains random primers and oligo(dT) in an unique ratio which works best for short transcripts. If you are looking for very large transcripts OR gene specific cDNA synthesis then we recommend to use DX/DT's cDNA Synthesis Kit - R6201/R6202.

PCR STEP

We recommend to use DX/DT LEO PRIME (R8220/ R8221) for below PCR assays.

Components	20µL reaction	50µL reaction	Final Concentration
Template DNA/cDNA	1 µL	2 µL	5ng - 100 ng
Forward Primer (10µM)	0.5 µL	1 µL	0.1 - 1µM
Reverse Primer (10µM)	0.5 µL	1 µL	0.1 - 1µM
LEO PRIME MasterMix (2X)	10 µL	25 µL	1X
Nuclease Free Water	Upto 20 µL	Upto 50 µL	

PCR Conditions

Step	Temperature	Time	Cycle
Initial denaturation	95 ℃	2 minutes	1
Denaturation	95 ℃	20-30s	25 - 45 Cycles
Annealing (Tm of your primers)	℃	20-30s	
Extension	72 ℃	1 minute/kb	
Final Extension	72 ℃	2 - 5 minutes	1
Hold, if required.	2 - 8 ℃	variable	1

Any Help / Contact

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Scan the QR Code to read on trouble-shooting guide