Differentiating Research

Product Details

Phi-Script™ 1st Strand cDNA Synthesis Kit

Product Overview

Phi (ϕ) Script ™ 1st strand cDNA synthesis contains PhiScript reverse transcriptase manufactured by dx/dt. It'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. Good cDNA yields can be obtained when reverse transcribed at 42-60℃.

Catalog Details

R6201	20µlX50 Reactions
R6202	20µlX250 Reactions

Each Pack Contains

- Extrapure dNTPs (10µM Each)
- Random Primers (50µM)
- Oligo(dT) (50µM)
- RNase Inhibitor (40U/µL)
- PhiScript RT (200U/µL)
- 5X RT Reaction Buffer
- DTT (100mM)
- Nuclease Free Water

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. It is important to have intact RNA specially when longer, full length cDNA is expected. Make sure to have all the tips, plastic wares, other consumables and working area RNAse Free.

	Full length cDNA synthesis		Gene Specific cDNA Synthesis	
Components	**20µL Reaction**	**Target**	**20µL Reaction**	**Target**
Total RNA Template	variable	≤ 2 µg	variable	≤ 2 µg
dNTP Mix (10mM Each)	1 µL	0.5 mM Each	1 µL	0.5 mM Each
Either Random Primers (50µM Stock)	1 µL	upto 200 ng - Random primers	0	NA
OR Oligo(dT) primers (50µM Each)		upto 500 ng - (oligo-dT)	0	NA
OR Both 1µL Each				
Gene Specific Primer(10µM Stock) (**Reverse primer ONLY**)	0	NA	1 µL	2 - 20pmoles
Total Volume	X µL		X µL	

PRE - RT STEP

Mix all the above componens (1. RNA, 2. dNTP, 3. Random primers OR oligo(dT) or both) and heat it up at **70℃ for 5 minutes.** This step will remove secondary structures of RNA. After this, keep the vial on ice **immediately** at least for 1 minute. For **gene specific assays,** pre-RT step is optional, may not be essential.

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RT STEP

Perform the following reaction on ice.

Components	20µL reaction	Target Final Concentration
Pre - RT mixture	X µL	
DX/DT Rnase Inhibitor (40U/µL)	1 µL	10 - 40U
5X ϕScript™ Reaction Buffer	4 µL	1X
DTT (100mM Stock)	1 µL	Upto 2 - 10mM
ϕScript™ Reverse Transcriptase (200U/µL)	1 µL	upto 3 µL
Nuclease Free Water	upto 20µL	

- Incubate the above mixture at 42℃ for 30 - 60 minutes. Increase the reaction mixture temperature upto 55-60℃ for **gene specific assays**. If you are using random primers, then first incubate 2**5℃ for 10 minutes** prior to 42℃.
- Inactivate RT by heating at 85 ℃ for 10 minutes.
- Store the cDNA samples at -20℃ for further analysis. This cDNA now can be used as PCR template for amplification.

PCR STEP

We recommend to use DX/DT LEO PRIME Mastermix (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