Differentiating Research

Product Details

5X REACTION BUFFER FOR PHISCRIPT™ REVERSE TRANSCRIPTASE

Product Overview

DX/DT Reverse Transcription Buffer (5X) is a RNase-free, high-performance reaction buffer optimized for super-fast and efficient cDNA synthesis. Designed for reverse transcriptase enzymes, it ensures robust RNA-to-cDNA conversion even from low-quality or structured RNA. The buffer provides an ideal environment for one-step RT-PCR and qRT-PCR, supporting high RNA tolerance for challenging templates. With enhanced stability and efficiency, it enables rapid and accurate reverse transcription, making it perfect for gene expression analysis, viral RNA detection, and NGS applications. DX/DT Reverse Transcription Buffer (5X) ensures reproducibility, sensitivity, and superior performance in all RT-based assays.

Catalog Details

R6110-5	5 ml
R6110-25	25 ml

Storage

- 20 ℃

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
RNA Template	variable	1 µg	variable	1 µg
dNTP Mix (10mM Each)	1 µL	0.5 mM Each	1 µL	0.5 mM Each
Random Hexamers (50µM Stock)	1 µL	upto 200 ng (hexamers)	0	NA
OR Oligo (50µM Each)		upto 500 ng (oligo-dT)	0	NA
OR Both				
Gene Specific Primer(10µM Stock) (**Reverse 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. hexamer 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.

Differentiating Research

Product Details

5X REACTION BUFFER FOR PHISCRIPT™ REVERSE TRANSCRIPTASE

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) *(Optional)*	1 µL	10-40U
5X Reaction Buffer for RT	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 to 50-55℃ for **gene specific assays**. If you are using random hexamers, then first incubate 25**℃ 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) for below PCR assays (End Point PCR) or Polaris Mastermix (R2220) for qPCR applications

Components	20µL reaction	50µL reaction	Final Concentration
Template DNA/cDNA	1 µL	2 µL	1ng - 250 ng
Forward Primer (10µM)	0.8 µL	1 µL	0.1 - 1µM
Reverse Primer (10µM)	0.8 µL	1 µL	0.1 - 1µM
Extremo 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 Technical Help ?

Scan the QR Code to read on trouble-shooting guide

CONTACT DETAILS

info@dxbidt.com | www.dxbidt.com | +91-7349708807