amfiRivert Platinum One cDNA Synthesis Platinum Master Mix (4X)

R6100

Storage

Store at -20°C.

Contents

- Product Manual
- ***amfiRivert*** Platinum One cDNA Synthesis Platinum Enzyme Mix (4X)
- Water, DEPC treated for RNA application

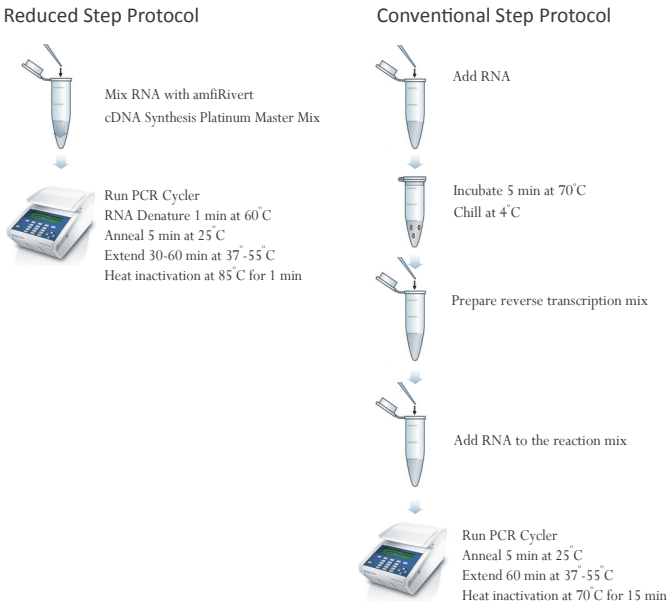
Shipping Condition

Ship with ice pack and dry ice.

Introduction

amfiRivert cDNA One cDNA Synthesis Platinum Master Mix (4X) is specially de -signed for high-yield Reverse transcription system that all provides components needed for cDNA synthesis. The master mix produces excellent results in both real-time and conventional RT-PCR. ***amfiRivert*** Platinum One cDNA Master Mix (4X) is fully active at 45-55 ℃, providing the higher cDNA yields and successful cDNA synthesis for diffucult RNA transcription. ***amfiRivert*** Platinum One cDNA Synthesis Master Mix (4X) includes an optimized primer mix which results in the generation of first-strand cDNAs from an entire transcript without the end-bias observed with typical oligo (dT)n or random hexamer primers. This mixed primer strategy over-comes variability in real-time PCR gene expression analysis that can result from using different individual primers. For convenience, ***amfiRivert*** Platinum One cDNA Synthesis Master Mix (4X) contains a ***amfiRivert*** Reverse Transcriptase, RNase Inhibitor Plus, $MgCl_2$ and dNTP Mixtures, and optimized primer mix ($Oligo(dT)_{18}$/Random Hexamer) minimizing extra optimization an pipetting steps.

Schematic Overview



Protocol-Reduced Step Protocol

Step 1. Prepare RT Reaction Mix

Table 1: Prepare Reverse Transcription Mix

Components	Per 20 ul reaction
Total RNA (or mRNA)	10pg - 5ug (10 pg - 500 ng)
amfiRivert Platinum One cDNA Master Mix	5 ul
DEPC treated water or PCR grade water	Up to 20 ul

Step 2. Perform Reverse Transcription Reaction

Table 2: Perform RT reaction with following cycling protocol

Step	Duration and Temp
RNA Denaturation (optional)	1 min at 60°C
Annealing	5 min at 25°C
Extend	30-60 min at (37-55°C)
RT Inactivation	1 min at 85°C
Hold	4°C

Step 3. Perform Real-Time PCR using synthesized cDNA

Add 1-2 ul of cDNA to 20 ul of real-time PCR reaction and perform real-time PCR.

Notes:

- Annealing conditions may require optimization.
- First-strand cDNA Synthesis occurs during the extension step. The extention temperature may require optimization.
- Extension time may be determined based on target gene abundance.
- Optimum cDNA dilution must be determined based on target gene abundance and qPCR chemistry.
- For RNA inputs 1 ug to 5 ug : cDNA generated with these must be diluted at least 10-fold in 10 mM TE buffer(pH 8.0), or PCR-grade water prior to use in qPCR.
- For input RNA less than 1 ug: cDNA generated with this can be used directly in qPCR.

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Protocol-Classical Step Protocol

Step 1. Prepare RNA Sample

Table 1: Prepare Reverse Transcription Mix

Components	Per 20 ul reaction
Total RNA (or mRNA)	10pg - 5ug (10 pg-500 ng)
DEPC Treated Water or PCR grade water	up to 15, (4X) ul

Step 2. Denature RNA and Hold

Table 2: Perform RNA denaturation

Step	Duration and Temp
RNA Denaturation	5 min at 70°C
Hold	4°C

Step 3. Prepare RT Reaction Mix

Table 3: Prepare Reverse Transcription Mix

Components	Per 20 ul reaction
Denatured RNA sample	15 ul
amfiRivert Platinum One cDNA master mix	5 ul
Total	20 ul

Step 4. Perform Reverse Transcription Reaction

Table 4: Perform RT reaction with following cycling protocol

Step	Duration and Temp
Annealing	5 min at 25°C
Extension	30-60 min at (37-55°C)
RT Inactivation	15 min at 70°C
Hold	4°C

Step 5. Perform Real-Time PCR using synthesized cDNA

Add 1-2 ul of cDNA to 20 ul of real-time PCR reaction and perform real-time PCR.

Troubleshooting

Problem	Possible Cause	Solution
Low (or no) yield cDNA product	Enzymes are inactivated due to incorrect storage.	Use the positive control to test performance of the enzymes.
	Template RNA is poor quality and/or degraded.	RNA purity and integrity is essential for synthesis and quantification of cDNA. Always assess the integrity of RNA prior to cDNA synthesis. Perform gel analysis of the RNA to determine the RNA quality. The A260/A280 ratio should be >1.7. SDS, NaCl, heparin and guanidine thiocyanate from RNA purification methods can interfere with RT-PCR. Reduce the volume of RNA, perform additional purification steps or change purification method to avoid interference. Use RNase-free reagents and equipment. Use freshly prepared RNA. Multiple freeze/thaw cycles of the RNA sample and synthesized cDNA is not recommended.
	Insufficient template quantity.	Increase the amount of template RNA in the reaction. (up to 1000 ng)
	GC-rich template.	If RNA template is GC-rich or is known to contain secondary structures, the temperature of the reverse transcription reaction can be increased up to 56°C. If cDNA synthesis is performed at temperatures higher than 50°C, use 2 ul of cDNA in PCR reaction.
Unexpected bands after electrophoresis	The primer sequence is not specific to the desired target.	Redesign the primers.
	Genomic DNA contamination indicated by higher molecular weight product than expected.	Pretreat RNA with DNase I.

Related Products

Product Name	Cat No
Oligo dT (18)	O1024
PureXtract RNAsol, Trizol equivalent	R6101
amfiRivert cDNA Synthesis Platinum Master Mix	R5600
RNazor, Rnase Decontamination Solution	R5800
RNazor, RNase Decontamination Solution	R7000
Water, DEPC Treated	W0805

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