amfiRivert cDNA Synthesis Platinum Master Mix

R5600

Storage

Store at -20°C.

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Introduction

amfiRivert cDNA Synthesis Platinum Master Mix is specially designed for high-yield Reverse transcription system that is fully active at 40-55°C, providing increase specificity with Gene-Specific Primers(GSPs) and the highest cDNA yield of all RTs. amfiRivert cDNA Synthesis Platinum Master Mix 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) or random hexamer primers. This mixed primer strategy overcomes variability in real-time PCR gene expression analysis that can result from using different individual primers. For convenience amfiRivert cDNA Synthesis Platinum Enzyme Mix contains a amfiRivert Reverse Transcriptase and RNase Inhibitor Plus. amfiRivert cDNA Synthesis Platinum 2X Buffer Mix contains a fixed $MgCl_2$ and dNTP Mixtures, and optimized primer mix(Oligo dT(18)/ Random Hexamer) minimizing extra optimization and pipetting steps.

Schematic Overview

Reduced Step 20 Min Protocol

Mix RNA with amfiRivert cDNA Synthesis Platinum Master Mix

Run PCR Cycler
Anneal 1 min at 25°C
Extend 18 min at 50°- 55°C
Heat inactivation at 85°C for 1 min

Conventional Step Protocol

Add RNA

Incubate 5 min at 70°C
Chill at 4°C

Prepare reverse transcription mix

Add RNA to the reaction mix

Run PCR Cycler
Anneal 5 min at 25°C
Extend 60 min at 37°-55°C
Heat inactivation at 70°C for 15 min

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 cDNA Synthesis Platinum Enzyme Mix	1 ul
amfiRivert cDNS Synthesis Platinum 2X Buffer Mix	10 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 - 20 minutes.

Step	Duration and Temp
Annealing	1 min at 25°C
Extend	18 min at 50°C (50-55°C)
RT Inactivation	1 min at 85°C
Hold	4°C

Table 3: Perform RT reaction with following cycling protocol- 35 minutes.

Step	Duration and Temp
Annealing	1 min at 25°C
Extend	30 min at 50°C(50-55°C)
RT Inactivation	4 min at 75°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 9 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	9 ul
amfiRivert cDNA Synthesis Platinum Enzyme mix	1 ul
amfiRivert cDNA Synthesis Platinum 2x Buffer mix	10 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 (40-55°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

PureXtract RNAsol , Trizol equivalent	R6101-020
RNazor, RNase Decontamination Reagent, Spray bottle	R7000-025
gRazor, Genomic DNA Removal Enzyme Mix	R5800-100
Ethanol, DEPC Treated, 70% , For RNA Purification	E4600-050
DEPC Treated Water	W0805-010

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