amfiRivert II cDNA Synthesis Prime Master Mix (4X)

R5500

Storage

Store at -20°C.

Thaw 2X reaction Buffer at room temperature just prior to use and refreeze immediately.

Contents

- Product Manual
- ***amfiRivert II*** cDNA Synthesis Enzyme Mix, 100 units/ul
- ***amfiRivert II*** cDNA Synthesis 2X Reaction Buffer with oligo (dT)

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Shipping Condition

Ship with ice pack and dry ice.

Introduction

amfiRivert II cDNA Synthesis Enzyme Mix formulation is specially designed for high-yield Reverse transcription system that is fully active at 42-55 °C, providing increase specificity with Gene-Specific Primers (GSPs) and the highest cDNA yield of all RTs. ***amfiRivert II*** cDNA Sythesis Enzyme Mix composed of a unique blend containing Moloney murine leukemia virus reverse transcriptase (M-MLV RT). RNase Inhibitor, and hydrophobic thermo-stablizers, which produces fewer errors in the cDNA transcripts and increased full-length cDNAs. It can generate cDNA from 100bp to 17kb. ***amfiRivert II*** cDNA Synthesis 2X Reaction buffer contains a fixed $MgCl_2$, Oligo(dT), and dNTP Mixtures, minimizing extra optimization and pipetting steps.

amfiRivert II cDNA Synthesis 2X Reaction Buffer

100mM Tris-HCl (pH 8.3 at 25°C), 150mM KCL and 20mM DTT, 1mM dNTPs, 0.5ug of oligo (dT), 6mM $MgCl_2$.

Protocol

Prepare RNA Target

1. Use sterile, nuclear-free, thin-walled tubes, rechilled on ice.
2. For each 20ul reverse transcription (RT) reaction, combine

Components	Final Conc.	Volume
RNA template Total RNA or mRNA	 10pg - 5ug 10 pg - 500 ng	
Nuclease-free water to a final volume of		8ul

3. Incubate at 70°C for 5 minutes (option).
4. Quick-chill at 4°C for 5 minutes and hold on ice.

Prepare Reverse Transcription Mix

1. For each 20ul RT reaction, combine

Components	Final Conc	Voulme
amfiRivert II 2X Reaction Buffer	1X	10ul
amfiRivert II Enzyme Mix	200 units	2 ul
Final Volume RT Mix per 20ul reaction		12 ul

2. Vortex gently to mix.
3. Dispense 12ul aliquots into reaction tubes.

Note: We recommend keeping the RT reaction mix chilled on ice prior to incubation.

Add Template to the Reaction Mix

1. For each individual reaction, add 8ul of the appropriate template nuclease free-water mix to the 12ul reverse transcription. If necessary, overlay with nuclease-free mineral oil. The final volume for each individual reaction will be 20ul.

Note: Add the RNA template + nuclease free-water mix immediately prior to incubation.

Reverse Transcription

1. Anneal at 25°C for 5 minutes.
 Note: - 5 minutes, 25°C annealing step is suggested for using oligo (dT) and random hexamer.
 - This step can be skipped for using gene specific primers.
 - If you need to improve specificity, minimize nonspecificity, try annealing at a more elivated temperature.
2. Extend first strand for 10 minutes at 50°C. The extension temperature may be optimized between 37°C - 55°C. Increase the reaction temperature to 55°C for gene-sepcific primer, difficult templates or templates with high secondary structure.
3. Heat-inactivate the ***amfiRivert II*** cDNA synthesis Enzyme Mix by incubation at 70°C for 15 minutes.
 Note: - Annealing conditions may require optimization.
 - First-strand cDNA synthesis occurs during the extension step. The extension temperature may require optimization.
4. Analyze cDNA, proceed with PCR or store frozen.

Related Products

Description	Cat No
PureXtract RNAsol , Trizol equivalent	R6101
RNazor, RNase Decontamination Reagent, Spray bottle	R7000
gRazor, Genomic DNA Removal Enzyme Mix	R5800
Ethanol, DEPC Treated, 70% , For RNA Purification	E4600
DEPC Treated Water	W0805

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Tips

Critical Optimization Parameters

Avoiding ribonuclease contamination

Successful RT-PCR is dependent on the quality of the RNA, RNase are ubiquitous in the laboratory and in cells, and precautions should be taken to eliminate the risk of contamination. Pretreatment of reagents and equipment is recommended to avoid RNase contamination.

Glassware should be heated to 180°C for 60 mins or placed in 0.1% DEPC (Diethyl -pyrocarbonate) solution at 37°C for 10 mins with vigorously mixing followed by heating at 70°C for 1 hr or autoclaving at 120°C for 30 mins to eliminate the residual DEPC. Also note that DEPC should always be used in a fume hood. Plastic tubes should be autoclaved and rinsed in chloroform to inactivate RNases. Wear gloves when handling reagents, equipment, and samples. Pipettors should be wiped with 80% ethanol or isopropanol before RNA work. Reverse reagents and equipment exclusively for RNA work and store them separately from those potentially contaminated with RNases.

RNA template

The RNA template can be total RNA, messenger RNA or viral RNA. Use the highest purity RNA (A260/A280 ratio of 1.7 or higher) to detect amplified PCR fragments more efficiently. The RNA smaple should be DNA free and should be avoid of any RNase contamination. Aspetic conditions should be maintained.

Primers

In order to distinguish between amplification from RNA or genomic DNA, design primers from different exons that are separated by at least one intron. Genomic DNA amplification will generate a larger fragment than DNA amplication. It is very important that the primers be complementary to the template especially at the 3’ end. In addition, the G/C content should be approximately 40-60%. It is important to determine the proper annealing temperature for any pair of primers. Primer pair that exhibit similar melting temperatures and are completely complementary to the template are recommended. Use the formula below to estimate the melting temperature (T_m). In general, use an annealing temperature for the RT-PCR reaction that is 5°C less than the T_m.

$T_m(°C) = 2(N_A + N_T) + 4(N_G + N_C)$

N = The number of primer adenine(A), thymidine(T), guanidine(G) or cytosine(C) bases.

Cycling condition

The number of cycles ranges from 20 to 50. Note that if the template RNA is limited, increasing the number of cycles may increase nonspecific product yield rather than the desired product.

Troubleshooting

Problem	Possible Cause	Solution
No product detected	Enzymes are inactivated due to incorrect storage	Use the positive control to test performance of the enzymes.
	Mineral oil problem	High-quality, nuclease free mineral oil should be used.
	Primer annealing temperature is incorrect	Annealing temperature 5°C below the Tm is the optimal starting point.
	Primer sequence is incorrect	- Redesign primers to be more complementary to the target gene. - The 3’ end of the primers should be completely complementary to the template. - The primers should have a G/C content of 40-60%. - Check that the primer sequences are not self-complementary especially at the 3’ end. -A sense and anti-sense primer must be used.
	Annealing temperature is too high	Decrease temperature as necessary.
	Extension time is too short	Set extension time for at least 60s per kb of target length.
	Cycle number is too low	Increase cycle number.
	Template RNA is poor quality and/or degraded	- Perform gel analysis of the template 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 methods to avoid interference. -Use RNase-free reagents and equipment. - Prepare new template RNA.
	Insufficient template RNA	Increase the amount of template RNA in the reaction (up to 1,000ng).
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.

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