amfiRivert Reverse Transcriptase

A1202

Storage

Store at -20°C.

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- ***amfiRivert*** Reverse Transcriptase 5X Reaction Buffer

Storage

Ship with ice pack and dry ice.

Introduction

amfiRivert Reverse Transcriptase is provided in quantities sufficient for 100 first-strand cDNA synthesis reactions of 20ul each. ***amfiRivert*** Reverse Transcriptase enables robust, full-length cDNA synthesis for the reproducible analysis of rare or long messages. The ***amfiRivert*** Reverse Transcriptase can be used to reverse transcribe total RNA, poly(A)+ mRNA or synthetic transcript RNA templates.

amfiRivert Reverse Transcriptase 5X Reaction Buffer

250mM Tris-HCl (pH 8.3 at 25°C), 375mM KCl and 50mM DTT, 17.5mM $MgCl_2$

Quality Control Assays

First-Strand cDNA Synthesis

Reverse Transcription is performed on a mixture of RNA templates ranging in length from 0.5 to 10kb primed with oligo(dT). The ranging is compared to a reaction using a reference lot of the enzyme. Prominent bands of 8 different cDNA products are observed by gel electrophoresis and autoradiography.

Amplification

When 0.25 zeptomoles (approximately 100 copies) of 1.2kb Kanamycin Positive Control RNA is reverse transcribed at 42°C and amplified (40 cycles), the result is a clear, discrete 323bp DNA product as visualized on a agarose gel by ethidium bromide staining.

Protocol

Prepare RNA Target and Primer

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

Components	Volume
RNA template	up to 1ug
Primer	20pmol or 0.5ug
Nuclease-free water to a final volume of	5ul

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
Water, DEPC Treated		Xul
amfiRivert 2X Reaction Buffer	1X	4ul
dNTP mix (10mM each)	0.5mM	1ul
RNase Inhibitor Plus	1ul	20ul
Vortex the mixture *amfiRivert* Reverse Transctiptase		1ul
Final Volume RT Mix per 20ul reaction		15ul

2. Vortex gently to mix.
3. Dispense 15ul aliquots into reaction tubes.

Note:

- amfiRivert Reverse Transcriptase 5X Reaction Buffer contains a fixed $MgCl_2$ concentration of 3.5mM (1X). However, higher concentrations may be achieved by adding additional $MgCl_2$.
- We recommend keeping the RT reaction mix chilled on ice prior to incubation.

Reverse Transciption

1. Anneal at 25°C for 5 minutes.

Note:

- The 5 minutes, 25°C annealing step is suggested for using oligo dT and Random Hexamer.
- This step can be skipped for using gene specific primer.
- If you need to improve specificity, minimize nonspecificity, try annealing at a more elivated temperature.

2. Extend the first strand for 60 minutes at 42°C. The extension temperature may be optimized between 37°C-55°C.
3. Heat-inactivate the *amfiRivert* Reverse Transcriptase by incubating at 70°C for 15 minutes.
4. Analyze cDNA, proceed with PCR or store frozen.

Note:

- Annealing conditions may require optimization step. The extension

Related Products

Description	Cat No
dNTP mixture, 10mM each	D0610
$Oligo(dT)_{18}$	O1024
RNase Inhibitor Plus	R2808
RNazor, RNase Decontamination Reagent, Spray bottle	R7000
DEPC Treated Water	W0805

GenDEPOT PO Box 454 Barker, Tx 77413 tech@gendepot.com