AMV Reverse Transcriptase, 10units/ul

A1201

Storage

Store at -20°C. Avoid exposure to frequent temperature changes.

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Shipping Condition

Ship with ice pack and dry ice.

Introduction

AMV Reverse Transcriptase (AMV RT) catalyzes the polymerization of DNA using template DNA, RNA or RNA:DNA hybrides. It requires a primer (DNA primers are more efficient than RNA primers) as well as Mg^{2+} or Mn^{2+}. The enzyme possesses an intrinsic RNase H activity. Both nonionic detergents and sulfhydryl compounds stabilize the enzyme activity in vitro.

AMV Reverse Transcriptase 5X Reaction Buffer

The AMV Reverse Transcriptase 5X Reaction Buffer supplied with this enzyme has a composition of 250mM Tris-HCl (pH 8.3 at 25°C). 250mM KCl, 50mM $MgCl^{2+}$, 2.5mM spermidine and 50mM DTT.

Enzyme Storage Buffer

AMV Reverse Transcriptase is supplied in 20mM potassium phosphate (pH 7.2 at 4°C), 0.2% Triton X-100, 2mM DTT and 50% glycerol.
Source: Purified from avian myeloblastosis virus particles.

Unit Definition

One unit is defined as the amount of enzyme required to catalyze the transfer of 1 nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C. The reaction conditions are: 50mM Tris-HCl (pH 8.3), 8.75mM $MgCl_2$, 40mM KCl, 10mM DTT, 0.1mg/ml acetylated BSA, 1mM radiolabeled dTTP and 0.25mM poly(A):oligo(dT)

Quality control

Activity, SDS-PAGE/purity, DNase, RNase, endonuclease, first-strand cDNA synthesis.

Protocol

1. The following procedure uses 2ug of RNA. In a sterile Nuclease-free micro -centrifuge tube, add the primer to the RNA sample. Use 0.5ug primer/ug RNA in a total volume of <11ul in water. Do not alter the ratio of primer to template RNA. Heat to 70°C for 5 minutes. Chill the tube on ice for 5 minutes and centri -fuge briefly to collect the solution at the bottom of the tube.
2. Add the following components to the annealed primer/template in the order shown.

Components	Volume
AMV 5X Reaction Buffer	5 ul
dNTP mixture, 10mM each	2. 5 ul
RNase Inhibitor	40 units
Sodium pyrophosphate, 40mM (prewarmed to 42°C)	2.5 units
AMV RT	30 units
DEPC Treated Water to final volume	25ul

3. Mix gently by flicking the tube.
4. Incubate for 60 minutes at 42°C for oligo(dT) primers or at 37°C for random hexamer primers.
5. Place the reactions on ice.
6. Up to 10ul of an RT reaction containing AMV RT and the supplies AMV RT Reaction Buffer can be added to a 50ul PCR amplification reaction that uses *Taq* DNA Polymerase.

Realated products

Description	Cat No
dNTP mixtures, 10mM each	D0610
RNase Inhibitor Plus	R2808
Water, DEPC Treated	W0805

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Tips

Critical Optimization Parameters

Avoiding ribonuclease contaminiation

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

Glassware should be heated to 180°C for 60 min or placed in 0.1% DEPC (Diethyl-pyrocarbonate) solution at 37°C for 10 min with vigorously mixing followed by heating at 70°C for 1 hr or autoclaving at 120°C for 30 min 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, equipments and samples. Pipettors should be wiped with 80% ethanol isopropanol before RNA work. Reserve reagents and equipment exclusively for RNA work and store them separately from those potentially conta -minated 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 sample should be DNA free and should be devoid of any RNase contamination. Aseptic conditions should be maintained.

Primers

In order to distinguish between amplification from RNA or genomic DNA, design primers from different exons that are seperated by al least one intron. Genomic DNA amplification will generate a larger fragment than RNA amplification. 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 pairs that exhibit similar melting temperatures and are completely estimate the melting temperature (Tm). In general, use an annealing temperature for the RT-PCR reaction that is 5°C less than Tm.

$Tm\ (°C) = 2\ (N_A + N_T) + (N_G + N_C)$

N = the number of primer adenine(A), thymine (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 Guide

Problem	Possible cause	Suggestion
No product detected	Enzymes are inactivated due to incorrect storage	Use the positive to test performance of the enzymes
	Mineral oil problem	High-quality nuclease free mineral oil should be used
	Primer annealing temperature is incorrect	An 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 shoul 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 method 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 1000ng)
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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