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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