DNase I (RNase-free) (1000 U)

Cat. No.

RE120

Composition

Contents	Amount
DNase I (RNase-free) (1000 U)	1000 U
10 × DNase Buffer	1 mL

Storage Conditions

Long-term storage at -20°C for 2 years. It is recommended to store in aliquots and avoid repeated freezing and thawing.

Introduction

Deoxyribonuclease I, referred to as DNase I, derived from recombinant E. coli strains, is an endonuclease that can digest single-stranded or double-stranded DNA, does not contain RNase, and can be used for various RNA samples of purification.

DNase I hydrolyzes phosphodiester bonds to produce mono- and oligodeoxynucleotides containing 5'-phosphate and 3'-OH groups. The optimum working pH range is 7~8. The activity of DNase I depends on Ca^{2+} and can be activated by divalent metal ions such as Co^{2+}, Mn^{2+}, Zn^{2+}, etc. In the presence of Mg^{2+}, DNase I can randomly cut double-stranded DNA at any position; in the presence of Mn 2+, DNase I can cut DNA double-strands at the same position, forming blunt ends or 1~2 sticky ends with nucleotide overhangs.

Usage

1. Total RNA extraction kit: prepare RNA solution without DNA;
2. Directly used for DNA digestion in RNA solution.

Enzyme activity definition

- 37℃, 10 min, the amount of enzyme needed to completely decompose 1 μg of plasmid DNA.

DNase Ⅰ storage solution

- 50 mM Tris-Acetate(pH 7.5)，10 mM $CaCl_2$，50% (v/v) Glycerol.

Imporatant Notes

1. DNase I is sensitive to physical denaturation, please do not shake violently when mixing;
2. The enzyme should be stored in an ice box or on an ice bath when used, and stored at -20°C immediately after use.

Protocol (For reference only)

1．Trial with Jinsha Biology's RNA extraction series kits (column loading process)

a) Use RNase-free ddH2O to dilute 10 × DNase Buffer to 1 × DNase Buffer;

b) Preparation of DNase I working solution: take 2 μL DNase I + 28 μL 1 × DNase Buffer for each reaction, and gently pipette to mix;

c) Connect the RNA extraction series kit, the operation is as follows: Add 350 μL protein-free solution to the adsorption column, centrifuge at 12,000 rpm (~13,400×g) for 30-60 s, pour off the waste liquid in the collection tube, and put the adsorption column put back into the collection tube;

d) Add 30 μL of DNase I working solution to the center of the adsorption column, and place at room temperature for 15 minutes;

e) Connect the kit of RNA extraction series, the operation is as follows: Add 350 μL protein-free solution to the adsorption column, centrifuge at 12,000 rpm (~13,400×g) for 30-60 s, discard the waste liquid in the collection tube, and put the adsorption column put back into the collection tube;

f) Subsequent operations were carried out according to the kit instructions of the RNA extraction series until the final RNA molecules were obtained.

2．Direct processing of RNA solution

(1) Reaction system

Please use RNase-free centrifuge tubes and pipette tips, and prepare as follows:

Components	System
10 × DNase Buffer	10 μL
DNase I (RNase-free)	10 μL
RNA	As require
RNase-free ddH_2O	Up to 100 μL

(2) Reaction conditions

Temperature	Time
37℃	15~30 min
Add EDTA solution with a final concentration of 2.5 mM, mix well	
65℃	10 min