Tinzyme Co., Limited

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DNase I (Deoxyribonuclease I), Recombinant

Product Number: DI04

Shipping and Storage

Store at -20℃

Description

DNase I (Deoxyribonuclease I), Recombinant is an endonuclease that catalyzes to the same extent the degradation of both single- and doublestranded DNA randomly,and produces 5'-P terminal oligonucleotides. As protease activity has been eliminated, this enzyme is stable around its optimum neutral pH range. So, this enzyme is suitable for RNA preparation at neutral pH.

Source

Recombinant enzyme derived from non-animal host

Concentration

20U/μl

Applications

1. For digestion of template DNA without buffer exchange, after in vitro transcription with T7 or SP6 RNA Polymerase.
2. For nick translation with DNA polymerase I .
3. Making a DNA library for shotgun sequencing in the presence of Mn2+.
4. For foot-printing analysis of the interaction between DNA and protein.
5. For digestion of genomic DNA before RT-PCR.

Unit definition

One unit is the amount of the enzyme that increases the absorbance at 260nm by 0.001 per minute at 37℃, pH5.0, with calf thymus DNA as the substrate.

Purity

Ribonuclease activity is not detected after incubation of 1μg of 16S, 23S rRNA with 10 units of this enzyme for 4 hours at 37℃, pH7.5.

Storage Buffer

50mM Tris-acetate(pH7.5), 10mM $CaCl_2$, 50%(v/v)glycerol.

Reaction Buffer:

100mM Tris-HCl(pH7.5 at 25℃), 25mM $MgCl_2$, 1mM $CaCl_2$.

Note

As protease activity has been eliminated, this enzyme is stable around its optimum neutral pH range. The enzyme requires a divalent metal ion for its activity. It randomly produces nicks in double-stranded DNA in the presence of Mg^{2+}, but in the presence of Mn^{2+}, both strands of double stranded DNA are cleaved into fragments. The enzyme loses its activity reversibly with EDTA, and irreversibly by heat treatment at 80℃ for 10 minutes.

Protocol:

Digestion of genomic DNA in a sample for RNA-PCR

1. Prepare the following reaction mixture.

Total RNA	20~50μg
10×DNase I Buffer (supplied)	5μl
Recombinant DNase I (RNase-free)	0.5μl (10 units)
Ribonuclease Inhibitor	20 units
DEPC-treated water	up to 50μl

2. Incubate for 20~30 min. at 37℃.
3. Perform one of the following procedures to inactivate DNase I
   - 3.1. Heat treatment
     - 3.1.1. Add 2.5μl of 0.5 M EDTA, incubate at 80 ℃ for 2 min.
     - 3.1.2. Increase reaction volume to 100μl with DEPC treated water.
   - 3.2. Phenol/Chloroform extraction
     - 3.2.1. Mix 50μl of DEPC treated water and 100μl of phenol/ chloroform/isoamyl alcohol (25:24:1) together.
     - 3.2.2. Centrifuge at 12,000 rpm for 5 min. at room temperature, then transfer the upper layer to a new tube.
     - 3.2.3. Add equal amount of chloroform/isoamyl alcohol (24:1) and mix.
     - 3.2.4. Centrifuge at12,000 rpm for 5 min. at room temperature, then transfer upper layer to new tube.
4. Add 10μl of 3M sodium acetate and 250μl of chilled ethanol, and then mix. Keep it for 20 min. At -80℃.
5. Centrifuge at 12,000 rpm for 10 min. at 4℃. Remove the supernatant.
6. Wash the precipitate with chilled 70% ethanol. Centrifuge at 12,000 rpm for 5 min. at 4℃ and remove the supernatant.
7. Dry the precipitate.
8. Dissolve the precipitate in a suitable amount of DEPC-treated water.

Confirm the genomic DNA is removed completely by electrophoresis and measure the RNA concentration. When the genomic DNA is not removed completely, increase the amount of enzyme or extend reaction time.