BenzNuclease DNA and RNA Nuclease

Cat. # BEE-N3116

For Research Use Only

Description:

BenzNuclease is a recombinant form of Serratia macescens extracellular endonuclease produced in Escherichia coli cells using a proprietary process at ACROBiosystems. BenzNuclease is a homodimer with monomer molecular masses about 30 kDa. Two disulfide bonds found in the nuclease are crucial to its activity and stability. The enzyme is a non-specific nuclease with high specific activity, which degrades both single- and double-stranded nucleic acids in any form (single stranded, double stranded, linear, circular and supercoiled). It hydrolyzes internal phosphodiester bonds present between the nucleotides to 5'-phosphorylated oligonucleotides of 3-8 bases in length.

Application :

Its high intrinsic activity and broad substrate tolerance make the endonuclease an ideal tool in a variety of biotechnological and pharmaceutical applications: removal of nucleic acid from protein samples (Elimination of nucleic acids from recombinant proteins; Purification of protein fragments from inclusion bodies; Sample preparation in western blotting or two-dimensional gel electrophoresis) ; Viscosity reduction in protein extracts.

Effect of Conditions on Activity:

BenzNuclease is functional between pH 6 and 10 (optimal at pH8 - 8.5) , and from 0℃ to 42 ℃ (optimal at 35 ℃ - 42 ℃). Mg^{2+} (1-2 mM) is required for enzyme activity.

1 mM EDTA reduced the activity by 30% in the presence of 1 mM $MgCl_2$; 0.1 M EDTA eliminated all enzyme activity. In the presence of 1 mM $MgCl_2$, enzyme levels were reduced 75% by 0.1 M $CaCl_2$ or 1 M NaCl. Under standard assay conditions, 1 mM iodoacetate had no effect on the enzymatic rate, whereas 1 mM mercaptoethanol and maleic acid reduced the activity by only 5 to 10%. 10 mM p-Chloromercurybenzoate completely inactivates the enzyme, while 0.64 M beta-mercaptoethanol in the presence of 2 M urea causes only partial inactivation of the enzyme. 4 or 7 M Urea increases the enzyme activity.

Removal of BenzNuclease:

BenzNuclease contain no "Tag" and used in downstream processing can be removed by various purification methods according to the purification strategy for the target protein.

Unit Definition:

One unit will digest sonicated salmon sperm DNA to acid-soluble oligonucleotides equivalent to a ΔA260 of 1.0 in 30 min at pH 8.0 at 37 ℃, which corresponds approximately to complete digestion of 37 μg DNA. Note that 1 KU=1000 units.

Concentration:

≥250 U/μl

Purity:

>99% as determined by SDS-PAGE of reduced BenzNuclease .

SDS-PAGE:

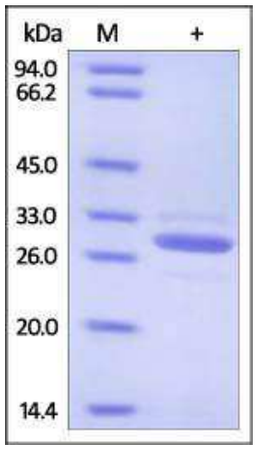

Fig.1 The purity of BenzNuclease was determined by reduced SDS-PAGE and staining overnight with Coomassie Blue.

Formulation:

Lyophilized in Tris HCl, pH 8.0, $MgCl_2$, and NaCl.

Reconstitution:

See Certificate of Analysis for reconstitution instructions and specific concentrations.

Storage:

Avoid repeated freeze-thaw cycles.
No activity loss was observed after storage at:
In lyophilized state for 1 year (4℃); After reconstitution under sterile conditions for 3 months (-70℃).

Activity Assay Procedure:

1. Reagents and solutions preparation

Reaction buffer*:
50 mM Tris-HCl, 1 mM $MgCl_2$, pH 8.0 （* In the case of extensive dilution before use, carrier protein such as 0.1 mg/ml HSA or BSA is generally recommended to avoid any enzyme loss from surface adsorption）

DNA Substrate:

1 mg/ml salmon sperm DNA is dissolved overnight at 4 ℃, in reaction buffer, and is then sonicated on ice to obtain a homogenous solution.

Enzyme:

Different dilution of nuclease with reaction buffer

Stop reagent:

Trichloroacetic acid (TCA)

2. Standard curve establishment

400 µl substrate + 100 µl enzyme of known activity

= 500 µl mixture

- Incubate the mixture at 37℃ for 30 min.
- Stop the reaction by addition of 400 µl cold TCA and incubate on ice for 10 min.
- Centrifuge at 8500 g for 5 min.
- Measure the absorbance of supernatant at 260 nm.
- Lot a standard curve with nuclease of known activities for each set of measurements.

3. Measurement of activity

The activity of any unknown nuclease can be determined from a single measurement by means of the standard curve. The specific activity of BenzNuclease is 1.3×10^6 ~ 1.5×10^6 unit/mg protein.

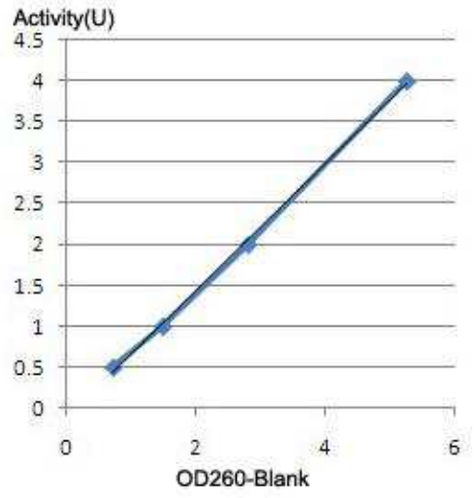

Fig 2. Standard curve of nuclease activity for BenzNuclease

References:

(1) Molloy, M.P., et al., 1998,Electrophoresis 19, 837-844.
(2) Herbert, B.R.,1998, Electrophoresis 19, 845-851.
(3) Benzonase® Brochure, 1999, Code No. W 220911, Merck KGaA, Darmstadt, Germany.
(4) Meiss, G., et al.,1995, Biochemistry,34, 11979–11988

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