



# SuperNuclease

**Catalog Number : SSNP01**

**Purity: > 95%      Unit: 10KU**

**Purity: > 95%      Unit: 25KU**

**Purity: > 95%      Unit: 50KU**

**Purity: > 95%      Unit: 500KU**

**FOR RESEARCH USE ONLY !**

## Description:

SuperNuclease is an extracellular *Serratia marcescens* endonuclease produced from genetically engineered *E. coli*. The enzyme is a nonspecific nuclease with high specific activity, which cleaves both RNA and DNA in any form ( single stranded, double stranded, linear, circular and supercoiled ) with similar rates. It hydrolyzes internal phosphodiester bonds present between the nucleotides. All free nucleic acids can be completely digested to 5'-monophosphate-terminated oligonucleotides of 3 to 8 bases in length. Under in vitro conditions, however, nuclease has been shown to prefer GC over AT-rich regions, as does DNase I. The nuclease is a homodimer with monomer molecular masses ranging from 25 to 30 kDa. Two disulfide bonds found in the nuclease are crucial to its activity and stability.

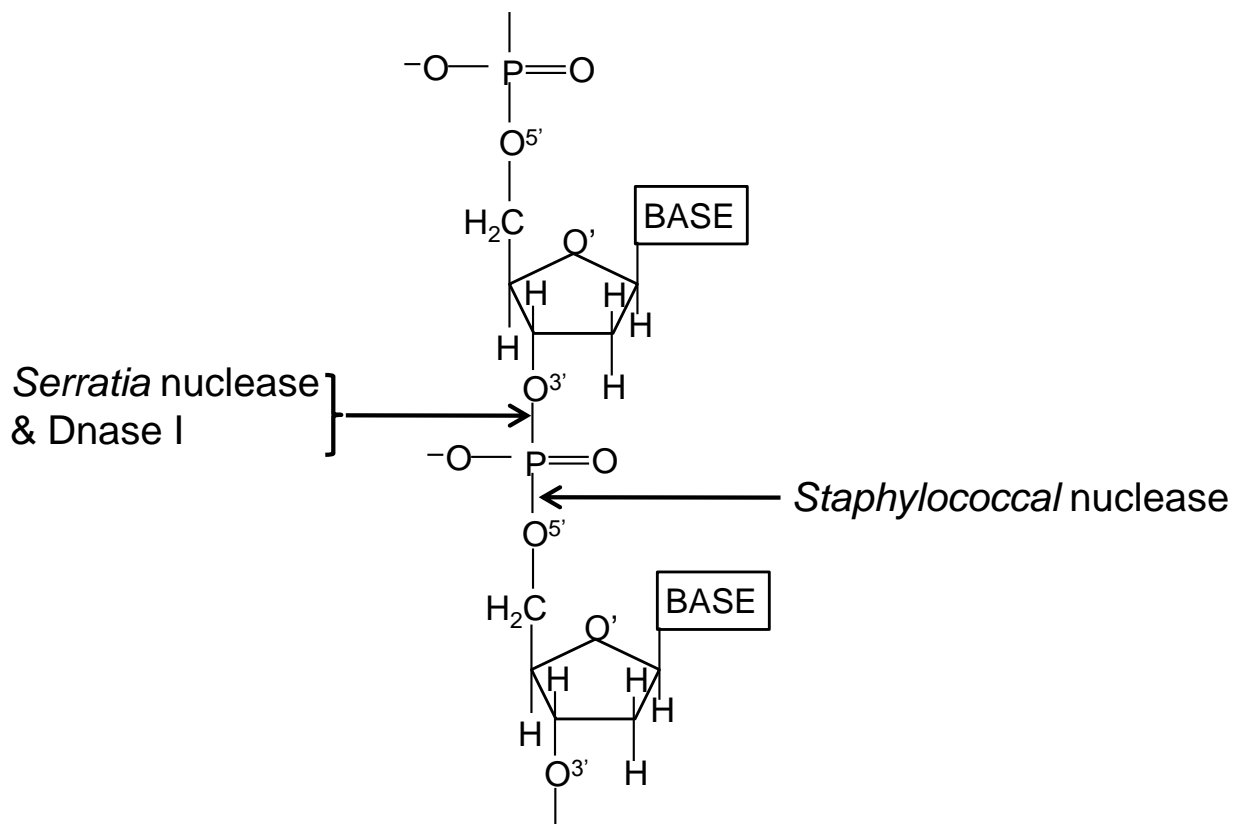


Fig 1. The cleavage sites for DNase I, Staphylococcal nuclease and *Serratia* nuclease on a polynucleotide substrate

## Effect of Conditions on Activity:

It requires  $Mg^{2+}$  or  $Mn^{2+}$  for activity and displays a broad pH range from 6 to 10 (optimal at 8 - 8.5) and a wide temperature optimum between 35°C and 44°C. Maximal activity obtained with 10 mM  $MgCl_2$  was three times that attained with 1 mM  $MnCl_2$ . 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-Chloromercuribenzoate 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.

## Applications:

Its high intrinsic activity and broad substrate tolerance make the endonuclease an ideal tool in a variety of biotechnological and pharmaceutical applications.

- Downstream processing
  1. Removal of nucleic acid contamination from purified proteins, commonly from recombinant DNA products.
  2. Reduction of viscosity for subsequent processing steps.
- Environmental protection
  1. A killer gene for the self destruction of microorganisms released into the environment.
- Novel applications
  1. Anti-viral agent; use for antiretroviral therapy; anti-tumor properties.

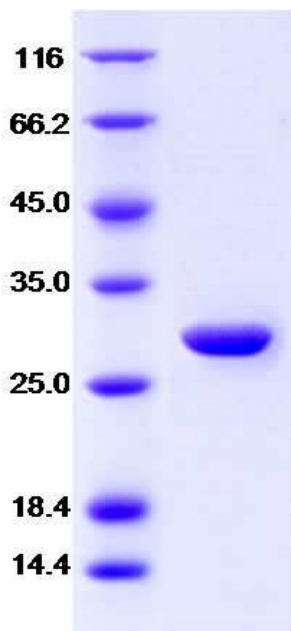
## Storage:

50mM Tris, 20mM NaCl, 2mM  $MgCl_2$ , pH 8.0 at -80°C

## Quality Assurance:

- **Purity**

The purity of each lot was determined by SDS-PAGE.



- **Activity Assay**

### Unit definition:

One unit of SuperNuclease is defined as the amount of enzyme that causes a  $\Delta A_{260}$  of 1.0 in 30min at 37°C, which corresponds approximately to complete digestion of 37  $\mu$ g DNA. Note that 1KU=1000 units.

### Procedure

#### 1. Reagents and solutions preparation

Reaction buffer: 50 mM Tris-HCl, 5 mM MgCl<sub>2</sub>, pH 8.5

Substrate: 1mg/ml salmon sperm DNA in reaction buffer

Enzyme: different dilution of nuclease with reaction buffer

Stop reagent: Trichloroacetic acid (TCA)

## 2. Standard curve establishment

400  $\mu$ l substrate + 100  $\mu$ l enzyme of known activity = 500 $\mu$ l mixture

1. Incubate the mixture at 37°C for 30min.
2. Stop the reaction by addition of 0.5 ml cold TCA and incubate on ice for 10 min.
3. Centrifuge at 8000g for 5 min.
4. Measure the absorbance of supernatant at 260 nm.
5. Plot a standard curve with nuclease of known activities for each set of measurements.

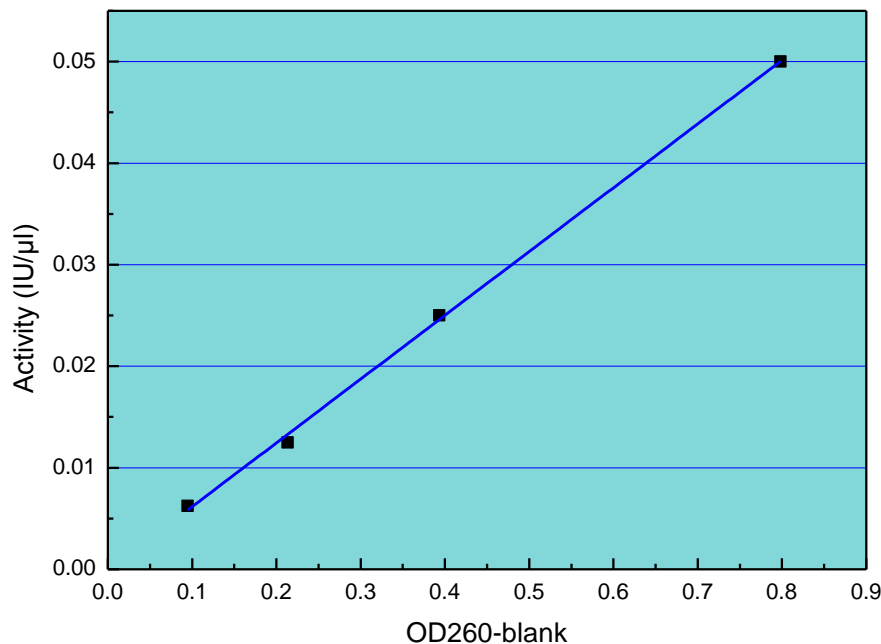


Fig 2. Standard curve of nuclease activity for SuperNuclease

## 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 SuperNuclease is  $1.3 \times 10^6 \sim 1.5 \times 10^6$  unit/mg protein.

## ● Absence of Protease Activity

Absence of protease activity for SuperNuclease is guaranteed by a highly sensitive and validated assay using an azo-casein as substrate. This eliminates the concern for SuperNuclease application in protein production process to yield high quantity and quality protein.

## Removal of SuperNuclease

SuperNuclease used in downstream processing can be removed by various purification methods according to the purification strategy for the target protein.

## Contact and Support

If you have any questions about the products and orders, please contact us:

E-mail: [Order@Sinobiological.com](mailto:Order@Sinobiological.com)

Tel: 86-10-5102-9968 ; Fax: 86-10-5102-9969

## References

- [1] Friedhoff P, Gimadutdinow O, *et al.* A procedure for renaturation and purification of the extracellular *Serratia marcescens* nuclease from genetically engineered *Escherichia coli*. 1994, Protein Expr Purif., 5(1):37-43.
- [2] Nestle M, Roberts WK. An extracellular nuclease from *Serratia marcescens* I . Purification and some properties of the enzyme. 1969, J Biol Chem. 10, 244(19):5213-5218.
- [3] Nestle M, Roberts WK. An extracellular nuclease from *Serratia marcescens* II. Specificity of the enzyme. 1969, J Biol Chem. 10, 244(19):5219–5225.
- [4] Michael J. Benedik, Ulrich Strych. *Serratia marcescens* and its extracellular nuclease. 1998, FEMS Microbiology Letters, 165: 1-13.
- [5] Timothy K.Ball, Yousin Suh and Michael J.Benedik. Disulfide bonds are required for *Serratia marcescens* nuclease activity. 1992, Nucleic Acids Research, Vol. 20, No. 19: 4971-4974.
- [6] M. Kunitz. Crystalline ribonuclease. 1940, The Journal of General Physiology: 15-32.
- [7] M. Kunitz. A spectrophotometric method for the measurement of ribonuclease activity. 1946, The Journal of Biological Chemistry: 563-568.
- [8] Christian B. Anfinsen, Robert R. Redfield, *et al.* Studies on the gross structure, cross-linkages, and terminal sequences in ribionuclease. 1953, The Journal of Biological Chemistry: 201-210.
- [9] George N. Eaves, Charles D. Jeffries. Isolation and properties of an excellular nuclease of *Serratia marcescens*. 1963, J. Bacteriol. Vol. 85: 273-278.
- [10] US Patent No. 5, 173, 418.
- [11] Filimonova M.N., Balaban N. P., Sharipova F. P. and Leshchinskaia I. B. Isolation and physico-chemical properties of homogenous nuclease from *Serratia marcescens*, 1980, Biokhimiia, 45: 2096-2104.