General Information

**Manufactured By:** Sino Biological Inc.

**Synonym:** Nuclease, Serratia marcescens’ extracellular endonuclease.

**Protein Construction:**
SuperNuclease is a recombinant Serratia marcescens’ extracellular endonuclease.

**Source:** Aequorea victoria

**Expression Host:** E. coli

QC Testing

- **Purity:** > 98%, as determined by SDS-PAGE.
- **Endotoxin:** < 0.05 EU/1000 units as determined by the LAL method.
- **Stability:** Samples are stable for up to twelve months from date of receipt at -70°C.

**Predicted N terminal:**
Three isoforms with different N terminal may be found from the compound—Sm1 (22D-266N), Sm2 (23D-266N) and Sm3 (25E-266N), the activity analysis shows that they were functionally equivalent.

**Molecular Mass:**
The SuperNuclease comprises 266 amino acids and has a calculated molecular mass of Sm1 (26708.2 Da), Sm2 (26591.8 Da) and Sm3 (26376.4 Da). The apparent molecular mass of SuperNuclease is approximately 26.5 KDa.

**SDS-PAGE:**
Fig. 1.

**Free of Protease Activity:**
Protease activity was monitored by azocasein assay (Fig. 2).

Usage Guide

**Shipping:**
Shipped at ambient temperature. The liquid or lyophilized enzyme is stable for at least 21 days when stored at 37°C (or ambient temperature).

**Formulation:**
SuperNuclease is lyophilized from sterile 50 mM Tris-HCl pH 8.0, 20 mM NaCl, 2 mM MgCl2, 5 % trehalose, 5 % mannitol, and 0.01 % Triton® ( Triton® is a trademark of Union Carbide, USA). Follow the instructions on the vial. Centrifuge the vial at 4°C before opening to recover the entire contents. Please contact us for any concerns or special requirements.

**Reconstitution:**
Follow the instructions on the vial. Centrifuge the vial at 4°C before opening to recover the entire contents. Normally, 25U/μL is recommended to be the final concentration.

**Storage:**
Store it under sterile conditions at -20°C to -80°C upon receiving for at least 12 months. Recommend to aliquot the protein into smaller quantities for optimal storage. Avoid repeated freeze-thaw cycles.

Protocol

**Large scale cell lysis treatment:**
1. Lysis buffer preparation:
The lysis buffer should be suitable for the protein as well as the downstream purification processes, and so on;
2. Resuspend cell plates in lysis buffer:
The ratio of lysis buffer (mL) against the gram of cell can be (10-20):1;
3. Add SuperNuclease (250 units to 1 g cells):
   It is well recommended to optimize the amount of SuperNuclease;
4. Lysate cell by mechanical or chemical methods on ice or at room temperature:
   Methods: Ultrasonic disruption, High Pressure Homogenizer, tissue homogenizer, and so on;
5. Obtain clear cell lysate supernatant by centrifugation at ~12,000 rpm for 0.5 hour.

Examples

1. **Viscosity Reduction of E. coli Lysate**
   E. coli (1.0 g) with a recombinant pET-28a construct was suspended in cell lysis buffer (50 mM Tris-HCl pH 8.0, 4 M Urea, 100 mM DTT, and 1% Triton X-100), and resulted in 1.0 g/mL. Then cell lysate was incubated with SuperNuclease at 4°C for 5 min. Then samples were centrifuged at 10,000 g for 1 min, and photographed (Fig.3).

2. **Salmon Sperm DNA Cleavage Assay**
   The substrate Deoxyribonucleic acid sodium salt from salmon testes (Sigma, Catalog # D1626) was diluted with assay buffer (50 mM Tris-HCl pH 8.0, 5 mM MgCl2, 100 μg/mL bovine serum albumin) into 1 mg/mL. Incubate the substrate with different units of SuperNuclease as well as other nucleases at 37°C for 30 min. The DNA fragment was analyzed by agarose gel electrophoresis, and photographed (Fig.4).

Fig. 3. A. With 25 U/mL SuperNuclease, B. Without SuperNuclease. Viscosity reduction assay shows that the SuperNuclease can reduce the viscosity of E. coli lysate.

Fig. 4. Comparison of the SuperNuclease and other nucleases in different amount of nuclease by plasmid DNA cleavage assay. *The DNase I's activity unit is decided by the DNase I's definition.

Protein Description

The SuperNuclease is a nonspecific nuclease with high activity, capable of completely digesting RNA and DNA (single stranded, double stranded, linear, circular and super coiled forms, that no fewer than five phosphate residues [1]) into 5'-monophosphate-digesting RNA and DNA (single stranded, double stranded, linear, circular and super coiled forms, that no fewer than five phosphate residues [1]) into 5'-monophosphate.

**References**