Catalog #	Aliquot Size
EN01-E311U-10	10 000 U
EN01-E311U-25	25 000 U
EN01-E311U-50	50 000 U

Endonuclease

Recombinant *Serratia marcescens* protein expressed in *E. coli*

Catalog # EN01-E311U

Lot # Y4244-13

Product Description

Recombinant tag-free Endonuclease (22-266) from *Serratia marcescens* was expressed in *E. coli*. The gene accession number is WP_164053777.

Alternative name (s)

Serratia marcescens nuclease, nuclease. The UniProt accession number is P13717.

Formulation

Recombinant protein stored in 50mM Tris (pH 8.0), 2mM $MgCl_2$, 20mM NaCl, and 50% Glycerol.

Storage and Stability

Aliquot protein into smaller quantities after centrifugation and store at –20°C. Avoid repeated handling and multiple freeze/thaw cycles.

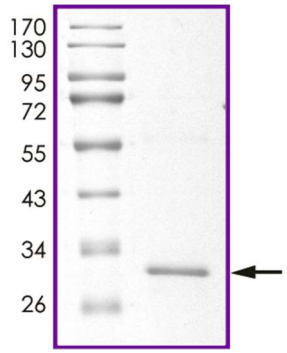
Scientific Background

Serratia marcescens endonuclease is a nonspecific nuclease with little sequence selectivity that can cleave single stranded, double stranded, linear, circular, or supercoiled DNA and RNA substrates (1, 2). The enzyme completely digests nucleic acids to 5'-monophosphate terminated oligonucleotides 2-5 bases in length and is effective over a wide range of operating conditions. It is extracellularly secreted by the *Serratia marcescens* bacteria and is thought to contribute to its virulence by scavenging nucleic acid from the extracellular environment (3). The enzyme may be used for various biotechnological applications including removing nucleic acids from protein samples, prevention of cell clumping, and replacement of sonication or mechanical shearing of DNA.

References

1. Meiss G, Gast FU, Pingoud AM. The DNA/RNA non-specific Serratia nuclease prefers double-stranded A-form nucleic acids as substrates. J Mol Biol. 1999 May 7;288(3):377-90. PMID: 10329148.
2. Meiss G, Friedhoff P, Hahn M, Gimadutdinow O, Pingoud A. Sequence preferences in cleavage of dsDNA and ssDNA by the extracellular Serratia marcescens endonuclease. Biochemistry. 1995 Sep 19;34(37):11979-88. PMID: 7547935.
3. Benedik MJ, Strych U. Serratia marcescens and its extracellular nuclease. FEMS Microbiol Lett. 1998 Aug 1;165(1):1-13. PMID: 9711834.

Purity



SDS-PAGE gel image
The purity of Endonuclease was determined to be **>90%** by densitometry.
Observed MW **~30 kDa**
Calculated MW **26.9 kDa**

Activity

The specific activity of endonuclease was determined to be **100 units/µl** as per the activity assay protocol.

Unit Definition:

One unit of endonuclease is defined as the amount of enzyme that causes a change in absorbance at 260nm of 1.0 in the time of 30 minutes, which corresponds to complete digestion of 37 mg of DNA. Standard reaction conditions are 0.1 mg/ml sonicated DNA substrate in 50mM Tris-HCl pH8.0, 0.1mg/ml BSA, 1mM $MgCl_2$. incubated at 37°C; measured as perchloric acid soluble digestion product.

Endonuclease

Recombinant *Serratia marcescens* protein expressed in *E. coli*

Catalog #	EN01-E311U
Lot #	*Y4244-13*
Purity	>90%
Activity	100 units/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Aliquot product into smaller quantities after centrifugation and store at –70°C. Avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Protein Extract Sample Preparation Protocol

Principle: The following protocol outlines the preparation of protein extract samples using Endonuclease (Catalog #: EN01-E311U).

1. Treatment of tissue and cell lysate:

   Animal and plant tissues: Completely disrupt 30-50 mg tissues. Add 100-200 mL RIPA lysis buffer and 1 mL endonuclease. Mix well by vortex. Incubate at room temperature for 30 minutes.

   Mammalian cells: Resuspend 10^6-10^7 cells in 100 mL RIPA lysis buffer and add 1 mL endonuclease. Mix well by vortex. Incubate at room temperature for 30 minutes.

   ***E. coli* cells:** Centrifuge 500 mL cell culture at 8,000 rpm for 5 minutes. Discard supernatant and resuspend the cells in 100 mL lysis buffer and add 1 mL endonuclease. Mix well by vortex. Incubate at room temperature for 30 minutes.

2. After endonuclease treatment, centrifuge at 13,000 rpm for 10 minutes. Collect supernatant for following protein purification or analysis.

Endonuclease Activity Assay Protocol

Reaction Components

Endonuclease (Catalog #: EN01-E311U)

Active nuclease diluted with assay reaction buffer to 40U/ml and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of active nuclease for optimal results).

Perchloric acid solution (4%)

Dilute 70% perchloric acid (Sigma, Catalog#: 244252) with Milli-Q water to 4%.

Assay reaction buffer

Buffer components: 50mM Tris-HCl, pH 8.0, 1mM Magnesium Chloride and 0.1%(w/v) Bovine Serum Albumin

Substrate

Herring Sperm DNA (Promega, Catalog#: D1815) was diluted in assay reaction buffer to a final concentration of 1mg/ml.

Assay Protocol

Principle: The endonuclease assay is based on spectrophotometric reaction using herring sperm DNA as substrate. The enzyme digests herring sperm DNA into oligonucleotides of 2 - 5 base pairs in length. The production of these acid-soluble oligonucleotides leads to an increase in absorbance at 260 nm.

Unit Definition: One unit corresponds to the amount of enzyme required to produce a change in absorbance at 260 nm of 1.0 in the time of 30 minutes, under optimal conditions with excess substrate.

Step 1. Dilute 5µl of the sample with ice-cold assay reaction buffer to 15ml as enzyme solution.

Step 2. Pipette 2.5ml of substate buffer into a test tube and equilibrate at 37°C for about 5 minutes.

Step 3. Add 0.125ml of enzyme solution and incubate at 37°C.

Step 4. After 30 min, 45 min, and 60 min, pipette 0.5 ml each of the assay mixer into a 1.5ml of Eppendorf tube containing 0.5ml of ice-cold perchloric acid solution (4%).

Step 5. Mix and cool on ice for 60 min.

Step 6. Centrifuge the mixture at 13,200rpm for 6 minutes.

Step 7. Transfer supernatant to a cuvette and measure the optical density at 260nm against water (ODtest).

Step 8. Set up the blank control as outlined in steps 2 - 7, excluding the addition of the enzyme solution. Replace the enzyme solution with assay reaction buffer (ODblank).

Calculation of Specific Activity of Nuclease (U/µl)

Activity can be calculated by using the following formula:

$$\text{Volume activity(U/µl)} = \frac{\Delta OD(ODtest - ODblank) \times Vt \times df \times 30 \times 2}{t \times Vs \times 1000}$$

Vt: Total Volume (2.625 ml)
df: dilution factor (3000)
30: one unit is defined at 30 minutes
2: dilution factor of the measuring solution

t: reaction time
Vs: sample volume(0.125ml)
1000: conversion factor of ml to µl

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: Endonuclease **Catalog # EN01-E311U**

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name:	SignalChem Diagnostics Inc.
Street Address:	*110-13120 Vanier Place*
City, Prov. Postal Code:	*Richmond, BC, V6V 2J2*
Country	*Canada*
Fax:	604-232-4601
EMERGENCY PHONE:	604-232-4600

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** None.
- **Hazard Pictograms:** None.
- **Signal words:** None.
- **Hazard statements:** None.
- **Precautionary statements:** None.
- **Other hazards:** None known.

Article 3 – Composition/Information on Ingredients

Chemical Characterization: Mixture.
Description: This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	50%
Tris-HCL	2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride	1185-53-1	0.8%
NaCl	Sodium chloride	7647-14-5	0.1%
$MgCl_2$	Magnesium Chloride Hexahydrate	7791-18-6	0.04%
Protein	N/A	N/A	N/A

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breath in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Remove contact lenses, if present and easy to do so. Consult a physician.
- **After swallowing:** Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.

Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

SAFETY DATA SHEET

Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place at recommended temperature specified on the product datasheet. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**
  NA
- **Appropriate engineering controls:**
  Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**

Respiratory protection:
Use appropriate respirator if there is inadequate ventilation by following the government standards.

Hand protection:
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.

Eye/face protection:
Safety goggles with side-shields approved under appropriate government standards.

Skin/body protection:
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

Appearance: Colorless fluid.	**Danger of explosion:** Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	**Explosion limits:** Not available.
pH: Not available.	**Decomposition temperature:** Not available.
Melting point/freezing point: Not determined.	**Vapor pressure at 20 °C:** Not available.
Boiling point/Boiling range: >100 °C.	**Density:** Not determined.
Flash point: > 100 °C.	**Relative density:** Not determined.
Flammability (solid, gaseous): Not determined.	**Vapor density:** Not determined.
Ignition temperature: Not determined.	**Evaporation rate:** Not determined.
Auto-igniting: Product is not self-igniting.	**Solubility in / Miscibility with Water:** Fully miscible.

Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Not determined.
- **Hazardous decomposition products:** Not determined.

SAFETY DATA SHEET

Article 11 - Toxicological Information

- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
  Inhalation: No data available
  Ingestion: No data available
  Skin: No data available
  Eyes: No data available
- **Signs and Symptoms of Exposure:** No data available
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** No data available.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

Article 13 - Disposal Considerations

- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

Article 15 – Regulatory Information

- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.