

DESCRIPTION

Source *E. coli*-derived *S. marcescens* NucA protein
Met1-Asn266
Accession # P13717

Predicted Molecular Mass 28.9 kDa

SPECIFICATIONS

SDS-PAGE 29 kDa, reducing conditions

Activity Measured by its ability to hydrolyze DNA from salmon testes.
The specific activity is >130,000 pmol/min/ug, as measured under the described conditions.

Purity >90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 100 mM Tris, 1 mM MgCl₂, pH 8.5
 - r*S. marcescens* NucA (Catalog # 10038-NA)
 - Deoxyribonucleic acid (DNA) sodium salt from salmon testes (Millipore Sigma, Catalog # D1626), 5 mg/mL stock in deionized water
 - Coupling Enzyme: Recombinant Mouse Alkaline Phosphatase/ALPL (rmALPL) (Catalog # 2910-AP)
 - Malachite Green Phosphate Detection Kit (Catalog # DY996)
 - 96-well Clear Plate (Catalog # DY990)
 - Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

- Assay**
1. Dilute 1 M Phosphate Standard by adding 10 µL of the 1 M Phosphate Standard to 990 µL of Assay Buffer for a 10 mM stock. Continue by adding 10 µL of the 10 mM Phosphate stock to 990 µL of Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
 2. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
 3. Prepare a reaction mixture containing 0.16 mg/mL DNA and 4 µg/mL rmALPL in Assay Buffer.
 4. Dilute r*S. marcescens* NucA to 4 ng/mL in Assay Buffer.
 5. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
 6. Load 25 µL of 4 ng/mL r*S. marcescens* NucA into empty wells of the same plate as the curve. Include a Control containing 25 µL of Assay Buffer.
 7. Add 25 µL of reaction mixture to the wells, excluding the standard curve.
 8. Seal plate and incubate at room temperature for 20 minutes.
 9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
 10. Add 100 µL of deionized water to all wells. Mix briefly.
 11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
 12. Read plate at 620 nm (absorbance) in endpoint mode.
 13. Calculate specific activity:

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Phosphate released* (nmol)} \times (1000 \text{ pmol/nmol})}{\text{Incubation time (min)} \times \text{amount of enzyme (}\mu\text{g)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.

- Final Assay Conditions**
- Per Reaction:
- r*S. marcescens* NucA: 0.1 ng (0.0001 µg)
 - rmALPL: 0.1 µg
 - DNA: 4 µg

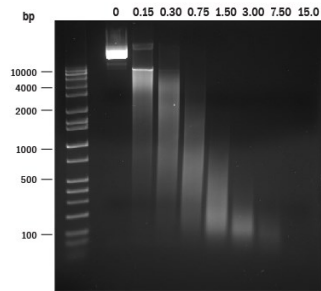
PREPARATION AND STORAGE

Shipping The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

- Stability & Storage** Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 6 months from date of receipt, -20 to -70 °C as supplied.
 - 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

Enzyme Activity



NucA (Benzonase) Digestion of DNA.

2 mg plasmid DNA was incubated with increasing amounts (ng/mL) of Recombinant *S. marcescens* NucA (Benzonase) (Catalog # 10038-NA) in 50 mM Tris (pH 8.0) and 1 mM MgCl₂ for 30 minutes at room temperature. Samples were analyzed by agarose gel electrophoresis and DNA was visualized by ethidium bromide staining.

BACKGROUND

Serratia marcescens endonuclease, also known as Benzonase, is a non-specific nuclease that degrades both single- and double-stranded nucleic acids, including DNA and RNA, but exhibits no proteolytic activity (1); therefore, it is ideal for the removal of nucleic acid contaminants from protein samples and applications where complete digestion of nucleic acids is desirable. NucA (Benzonase) is also commonly used in bioprocessing applications to reduce viscosity of samples caused by genomic DNA. The optimum pH for enzyme activity is found to be 8.0-9.2. It hydrolyzes internal phosphodiester bonds between nucleotides in nucleic acids to produce 5'-monophosphate oligonucleotides of 3-8 bases in length (2). The active enzyme is a homodimer with two disulfide bonds in each monomer that are crucial to the enzyme activity and stability (3). The absolute activity of the recombinant enzyme is measured by a phosphatase coupled assay (4), where the 5'-phosphate of oligosaccharides generated by the enzyme is further released by non-specific alkaline phosphatase and quantitated by Malachite reagents (5).

References:

1. Benedik, MJ and Strych, U. (1998) FEMS Microbiol Lett. **165**:1.
2. Nestle, M, *et al.* (1999) J. Biol. Chem. **274**:825.
3. Ball, T.K. *et al.* (1992) Nucleic Acids Res. **20**:4971.
4. Wu, Z.L. *et al.* (2011) Glycobiology **21**:727.
5. Van Veldhoven, P.P. and G.P. Mannaerts (1987) Anal. Biochem. **161**:45.