Micrococcal Nuclease

Description: Micrococcal nuclease is derived from Staphylococcus aureus and is a relatively non-specific endo-exonuclease. It is purified from a recombinant E. coli strain that digests double-stranded, single-stranded, circular and linear nucleic acids. The enzyme is active in the pH range of 7.0–10.0, with optimal activity at pH 9.2 for both RNA and DNA substrates. Cleavage preferences have been observed at sites rich in adenylate, deoxyadenylate or thymidylate (1). Both DNA and RNA are degraded to 3’ phosphomonomonucleotides and dinucleotides.

Source: An E. coli strain containing a genetic fusion of the micrococcal nuclease gene (Gene ID: 3238436) and the gene coding for maltose binding protein, or MBP. The micrococcal nuclease is cleaved from the fusion protein and purified away from MBP.

Applications:
- Degradate nucleic acids present in protein preparations
- In vitro translation (2)
- Reduce the viscosity of cell lysates during non-mechanical cell lysis preparation
- Chromatin structure analysis (3)
- Rapid RNA sequencing

Reagents Supplied with Enzyme:
- 1X Micrococcal Nuclease Reaction Buffer
- 100X BSA

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Notes:
This enzyme does not work in NEBuffer 1, 2, 3 or 4 due to the lack of Ca2+. Additional Ca2+ in NEBuffer only shows 10% activity. 1–5 mM Ca2+ is required for activity. The enzyme is active in the pH range 7–10 as long as salt concentration is less than 100 mM. Enzyme can be inactivated by addition of excess EGTA.

Unit Definition: (Kunitz Unit) One unit is defined as the amount of enzyme required to digest 1 µg of lambda genomic DNA in 15 minutes at 37°C, to the extent that the accumulation of low molecular DNA fragments (100–400 base pairs) disappears on a 1.2% agarose gel.

Notes: 10,000 Gel Units is approximately equal to 1,000 Kunitz Units.

Unit Assay Conditions:
- Kunitz Unit
- 1X Micrococcal Nuclease Buffer
- 0.1mg/ml BSA and 500 µg sonicated Salmon testis genomic DNA in a total volume of 500 µl.

Figure 1: Digestion of 1 µg of Lambda genomic DNA with Micrococcal Nuclease in a 3-fold dilution series. The amount of enzyme used in Lane 2 is defined as 1 gel unit. Lane M is the PCR Marker (NEB #N3234).
Quality Assurance: Free of detectable protease activity.

References: