Micrococcal Nuclease

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 prefers have been observed at sites rich in adenylate, deoxyadenylate or thymidylate (1).

Applications:
- Degrade 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:
10X Micrococcal Nuclease Reaction Buffer 100X BSA

Both DNA and RNA are degraded to 3’ phospho-mononucleotides 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.

Reaction Conditions: 1X Micrococcal Nuclease Reaction Buffer, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X Micrococcal Nuclease Reaction Buffer:
50 mM Tris-HCl
5 mM CaCl₂
pH 7.9 @ 25°C

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 release acid soluble oligonucleotides that produce an absorbance increase of O.D. 1.0 at 260 nm in 30 minutes at 37°C.

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).

molecular DNA fragments (100–400 base pairs) disappears on a 1.2% agarose gel.

Note: 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.
Quality Assurance: Free of detectable protease activity.

References: