INSTRUCTIONS

Micrococcal Nuclease

88216

2130.1

Number	Description
88216	**Micrococcal Nuclease,** ≥ 100 units/µL, 150µL, supplied in 10mM Tris•HCl pH 7.5, 50mM NaCl, 1mM EDTA, 50% glycerol Molecular Weight: 16,800 Source: *Staphylococcus aureus* **Storage:** Upon receipt store at -20°C. Product is shipped with dry ice.

Introduction

Thermo Scientific™ Micrococcal Nuclease is a stable liquid form of the enzyme derived from *Staphylococcus aureus*. Micococcal Nuclease exhibits exo- and endo-5'-phosphodiesterase activities against DNA and RNA.[1] This enzyme digests double-stranded, single-stranded, circular and linear nucleic acids. The highest activity is toward single-stranded nucleic acid substrates with preference for AT- or AU-rich regions. Enzymatic activity occurs at pH 7-10 and is strictly dependent on calcium for digestion of RNA and DNA substrates. Micrococcal Nuclease is suitable for removing nucleic acids from cell lysates, releasing chromatin-bound proteins and shearing chromatin for use in chromatin immunoprecipitation (ChIP) experiments.

Important Product Information

- Product is a liquid at -20°C. Keep enzyme on ice during use.
- Micrococcal Nuclease is dependent on Ca^{++} for activity. Avoid calcium chelators, such as EGTA, in reaction buffers.
- Enzyme is active at pH 7-10 with a salt concentration < 100mM. Optimal enzyme activity occurs at 37°C; however, the enzyme is active at room temperature. Monovalent metal ions, such as Na^{+} and K^{+}, will decrease activity. EGTA or heating to 65°C for 10 minutes will inactivate the enzyme.

Additional Materials Required

- Reaction Buffer: 50mM Tris•HCl pH 8.0, 5mM $CaCl_2$
- 10X Stop Solution (optional): 200mM EGTA, pH 8.0
- 100X BSA (optional): 10mg/mL in phosphate-buffered saline

Procedure

1. Add Micrococcal Nuclease to the sample diluted in Reaction Buffer.

   Note: If sample only contains nucleotides (i.e., no protein), add BSA to the Reaction Buffer at a final concentration of 0.1mg/mL.
2. Incubate reaction at 37°C until the nucleotides are degraded.
3. Optional: Stop reaction by adding EGTA, pH 8.0 to a final concentration of 20mM.

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Troubleshooting

Problem	Possible Cause	Solution
Minimal or no nucleotide degradation	No Ca^{++} present in the Reaction Buffer	Add a final concentration of 5mM $CaCl_2$ to the Reaction Buffer
	Reaction not complete	Incubate reaction longer
	Reaction temperature too high/low	Incubate reaction at 37°C
	Incorrect reaction pH	Maintain reaction at pH 7-10 using an appropriate buffer
	Presence of monovalent cations	Reduce monovalent cation concentrations to ≤ 100mM by dialyzing or desalting the sample

Related Thermo Scientific Products

78840 **Subcellular Protein Fractionation Kit**

69558 **Slide-A-Lyzer™ MINI Dialysis Units plus Float, 3.5K MWCO,** 10 units

89882 **Zeba™ Spin Desalting Columns, 0.5mL,** 25/pack

Reference

1. Cuatrecasas, P., *et al.* (1967). Catalytic properties and specificity of the extracellular nuclease of *Staphylococcus aureus. J Biol Chem* **242(7):**1541-7.