

OMNI Nuclease

(Recombinant)

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(*E. coli* strain with cloned *nucA* gene from *Serratia marcescens*)

Cat. No.	Size
E1120-01	20 000 units
E1120-02	100 000 units

Unit Definition:

One unit is defined as the amount of enzyme that causes a delta A260 of 1.0 in 30 min, which corresponds to complete digestion of 37 µg of Herring sperm DNA.

Storage Conditions: Store at -20°C (do not store at -80°C)

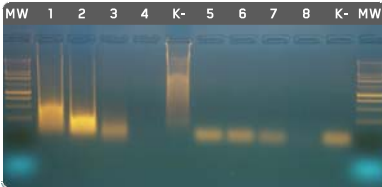


Figure 1: OMNI Nuclease activity test analyzed by 1% agarose gel electrophoresis. Lanes 1-4: 37 µg Herring sperm DNA, lanes 5-8: Torula Yeast RNA type VI, with increasing amounts of OMNI Nuclease units in lanes 1-4 and 5-8, respectively. MW: Molecular weight marker, K-: Control lanes without OMNI Nuclease addition. Incubation (digestion) at 37°C for 30 min.

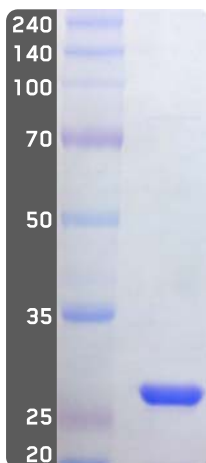


Figure 2: Purity check of OMNI nuclease on a 12% PAGE protein gel. Right lane: 5 µg OMNI nuclease. Left lane: Protein molecular weight marker (numbers indicate MW in kDa).

Applications:

- Viscosity reduction in bacterial, yeast and mammalian protein extracts
- Sample preparation for protein 2D electrophoresis
- Removal of nucleic acids contaminants from protein preparations

Description:

OMNI Nuclease catalyzes the removal of all forms of DNA and RNA (linear, circular, double and single stranded). The nuclease completely degrades nucleic acids to 5'-monophosphate terminated oligonucleotides with a length of 2 - 6 nt.

Notes:

1. OMNI Nuclease is very stable and can be diluted with 20 mM Tris-HCl (pH 8.0 @ 25°C), 20 mM NaCl, 2 mM MgCl₂ and stored at 4°C for many days without loss of activity.
2. Displays a broad pH tolerance (from 6 to 10) and has a broad temperature spectrum (0-44°C)
3. OMNI Nuclease is not recommended for purification of proteins that must be nuclease free. However, it can be separated from target molecules using chromatography, for example anion and cation exchange, hydrophobic interaction, hydroxyapatite or size exclusion. OMNI nuclease does not bind to Ni-NTA resin.
4. OMNI Nuclease is inhibited (app. 50% of activity) by:
 - monovalent cation concentrations (Na⁺, K⁺, etc.) > 50 mM
 - phosphate concentration > 20 mM
 - ammonium sulphate concentration > 25 mM

Instructions for use / Protocol:

For removal of nucleic acids:

Dilute substrate DNA or RNA in assay buffer (50 mM Tris-HCl pH 8.0, 1 mM MgCl₂, 100 µg/ml BSA) to 2 µg/µl. Incubate the substrate with different unit amounts of OMNI nuclease at 37°C for 30 min. Analyze by gel electrophoresis. To remove trace DNA/RNA use 2 U of nuclease to 1 µg of nucleic acids and extend incubation time to 24 h.

For viscosity reduction:

It is recommended to use 25 U per 1 ml of lysate. The ratio of lysis buffer (ml) to the gram of cells should be 1 g : 1-10 ml

Example:

1. Resuspend 5 g of *E. coli* (wet weight) in 25 ml of Tris-HCl pH 8.0, 1 mM EDTA (add lysozyme, protease inhibitors, if required).
2. Add MgCl₂ to a final concentration of 5 mM.
3. Add 2 µl (500 U) of OMNI Nuclease.
4. Perform disruption method of choice (Ultrasonic disruption, French press, etc.).
5. Obtain clear cell lysate supernatant by centrifugation at -12,000 rpm for 0,5 h and 4°C.

Storage Buffer:

20 mM Tris-HCl (pH 8.0), 20 mM NaCl, 2 mM MgCl₂, 50% Glycerol.

Assay Conditions:

50 mM Tris-HCl (pH 8.0, 1 mM MgCl₂, 100 µg/ml BSA, 37 µg of Herring sperm DNA. Incubation is at 37°C for 30 min in a reaction volume of 30 µl.

Quality Control:

All preparations are assayed for contaminating protease activity.

References:

1. Nestle M., Roberts W.K., (1969) *J. Biol. Chem.* 244, 5219-5225
2. Friedhoff P., Gimadutdinov D., (1994) *Protein Expr.Purif.* 5, 37-43
3. Meiss G., Friedhoff P., (1995) *Biochemistry* 34, 11979-11988