Manual

Omnicleave Endonuclease

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Omnicleave™ Endonuclease is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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GENOMIC ANALYSIS BY LGC

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1. Introduction

OmniCleave Endonuclease degrades single- and double-stranded DNA and RNA to di-, tri- and tetranucleotides. The enzyme is extensively purified from a recombinant strain of *E. coli.* OmniCleave Endonuclease is especially useful in protein purification for decreasing the viscosity of cellular lysates caused by nucleic acids. Removal of the nucleic acids improves the subsequent manipulation and yield of the purified protein. In addition, the enzyme is useful for removing host DNA and RNA in phage DNA preparations.

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part number	Volume
OmniCleave Endonuclease	50,000 Units	OC7850K	OmniCleave Endonuclease (200 U/µl)	E0051-200D1	250 µL
			OmniCleave Storage & Dilution Buffer	SS000250-D1	5 mL

3. Product specifications

Storage: Store only at -20 °C in a freezer without a defrost cycle.

Storage buffer: OmniCleave Endonuclease is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT) and 0.1% Triton® X-100 (Rohm & Haas).

Dilution Buffer: A 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM DTT and 0.1% Triton X-100.

Unit definition: One unit (~1 ng/U) of OmniCleave Endonuclease converts 1.0 OD_{260} (~50 µg) of sonicated salmon sperm DNA to acid-soluble nucleotides in 30 minutes at 37°C under the recommended assay conditions.

Quality control: RThe enzyme is function-tested in a reaction containing 50 mM Tris-HCl (pH 8.0), 1 mM $MgCl_2$ and 1 mg/mL sonicated salmon sperm DNA.

Contaminating activity assays: OmniCleave Endonuclease is free of detectable protease activity.

Purity: OmniCleave Endonuclease is ≥95% pure as judged by SDS-PAGE.

4. Enzyme characteristics

pH optimum: 8-9. Approximately 20% of optimal activity is retained between pH 6-10.

Reaction temperature: The enzyme is active up to 40 °C.

Divalent cations: The enzyme requires magnesium and is fully active between 1-10 mM magnesium. Activity decreases to 30% of maximum with the substitution of 1-10 mM manganese for magnesium.

Monovalent cations: None are required for optimal activity and the enzyme will retain approximately 50% activity in the presence of up to 100 mM sodium or potassium ions.

Detergents and chaotropes: The enzyme retains at least 50% activity in the presence of up to: 1% Triton X-100, 0.75% deoxycholate, 0.1% SDS and 6 M urea.

5. Protocol for decreasing the viscosity of cellular lysates

The following protocols result in >99% digestion of polymeric nucleic acids, which is sufficient for decreasing the viscosity of cellular lysates. For complete digestion of any residual nucleic acids, more nuclease or longer digestion times may be necessary.

Protocol for larger scale protein preparations (>1 gram of cells)

1. Pellet the cells. These cells can either be previously frozen or prepared fresh.
2. Suspend the cells in 50 mM Tris-HCl (pH between 8-9) containing between 1-10 mM $MgCl_2$ and ≤ 100 mM NaCl. If these buffer conditions are incompatible with subsequent manipulations, add more OmniCleave Endonuclease to compensate for sub-optimal activity.
3. Add 1,000 units of OmniCleave Endonuclease for each gram of cells used. The enzyme is more effective if added before lysis of the cells.
4. Lyse the cells by the method of choice. This is most easily done by adding 60,000 units of Ready-Lyse™ Lysozyme Solution for each gram of cells, incubating the cells for 10 minutes at room temperature and adding 0.1% deoxycholate.
5. Incubate the lysate at room temperature for 30 minutes to degrade the nucleic acids.
6. At the end of the incubation time, the viscosity of the lysate should be reduced to the point that the lysate is essentially nonviscous.
7. If desired, add 20 mM EDTA to chelate the magnesium.
8. Protein preparations may be used directly or further purified.

Protocol for smaller scale protein preparations (≤1 gram of cells)

In this protocol the amount of OmniCleave Endonuclease is determined using OD_{600}.

1. Determine the optical density of the cell culture at 600 nm and multiply the optical density by the original volume of the cell culture.
2. Pellet the cells by centrifugation.
3. Suspend the cells in 50 mM Tris-HCl (pH between 8-9) containing between 1-10 mM $MgCl_2$ and ≤100 mM NaCl. If these buffer conditions are incompatible with subsequent manipulations, add more OmniCleave Endonuclease to compensate for sub-optimal activity.
4. Add 10 units of OmniCleave Endonuclease for each optical density unit of the original culture, as determined in Step 1. OmniCleave Endonuclease is more effective if added before lysis of the cells..
5. Lyse the cells by the method of choice. This is most easily done by adding 600 units of Ready-Lyse Lysozyme Solution for each OD_{600}, incubating the cells for 5 minutes at room temperature and adding 0.1% deoxycholate.
6. Incubate the lysate at room temperature for 10 minutes.
7. At the end of the incubation time, the viscosity of the lysate should be reduced to the point that the lysate is essentially nonviscous.

6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com

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