

Product Contents

Mung Bean Nuclease:

Part No.	Size (units)
M431A	2,000

10X Reaction Buffer (M432A): When the Mung Bean Nuclease 10X Reaction Buffer, provided with this enzyme, is diluted 1:10, it has a composition of 0.03M sodium acetate (pH 5.0), 0.05M NaCl and 1mM ZnCl₂.

Enzyme Storage Buffer: Mung Bean Nuclease is supplied in 10mM Tris-HCl (pH 7.5), 50mM NaCl, 0.01% Triton® X-100 and 50% glycerol.

Source: Mung bean sprouts (*Phaseolus aureus*).

Unit Definition: One unit is defined as the amount of enzyme required to produce 1µg of acid-soluble material per minute at 37°C. The reaction conditions are: 30mM sodium acetate (pH 5.0), 50mM NaCl, 1mM ZnCl₂, 5% glycerol and 0.5mg/ml denatured calf thymus DNA. See the unit concentration on the Product Information Label.

Storage Temperature: Store at -20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Quality Control Assays

Contaminant Assay

Endonuclease/Nickase Activity: To confirm the absence of contaminating endonuclease/nickase activity, 1µg of lambda DNA/*Hind* III markers (Cat.# G1711) is incubated with Mung Bean Nuclease for 10 minutes at room temperature. The markers are then separated by electrophoresis on a 1% agarose gel and stained with ethidium bromide. Markers that have been incubated with ≥60 units of enzyme will remain as intact bands with a minimal amount of smearing.

Part# 9PIM431

Revised 8/05



Promega

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

PRODUCT USE LIMITATIONS, WARRANTY, DISCLAIMER

Promega manufactures products for a number of intended uses. Please refer to the product label for the intended use statements for specific products. Promega products contain chemicals which may be harmful if misused. Due care should be exercised with all Promega products to prevent direct human contact.

Each Promega product is shipped with documentation stating specifications and other technical information. Promega products are warranted to meet or exceed the stated specifications. Promega's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Promega makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO PROMEGA PRODUCTS. In no event shall Promega be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Promega products to perform in accordance with the stated specifications.

© 1997–2005 Promega Corporation. All Rights Reserved.

Triton is a registered trademark of Union Carbide Chemicals & Plastics Technology Corporation.

All specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Part# 9PIM431
Printed in USA. Revised 8/05

I. Description

Mung Bean Nuclease belongs to a class of enzymes that demonstrate a preference for single-stranded nucleic acids, lack sugar specificity and hydrolyze single-stranded substrates to products with 5'-phosphoryl and 3'-hydroxyl termini.

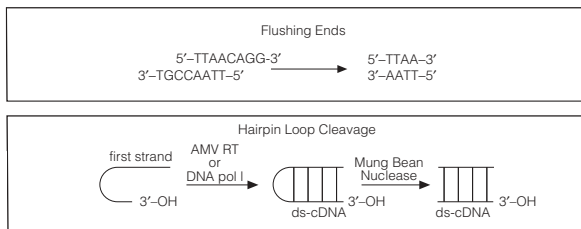


Figure 1. Mung Bean Nuclease catalyzes the generation of blunt-ended DNA and also cleaves hairpin loops to separate cDNA strands.

II. Digestion with Mung Bean Nuclease

A. Standard Applications

Mung Bean Nuclease catalyzes the degradation of single-stranded DNA and RNA endonucleolytically to yield 5'-phosphoryl terminated products. While the nuclease prefers ssDNA over dsDNA by 30,000-fold, at very high concentrations, such as 14 units/ μ g of DNA, the enzyme degrades double-stranded DNA preferentially from both ends (1–3). Mung Bean Nuclease has been used for transcript mapping studies (4,5), flushing staggered ends and for the separation of cDNA strands after synthesis with reverse transcriptase and DNA polymerase I (6).

B. Reaction Conditions for Flushing Ends of Double-Stranded DNA

Combine and mix 20 μ g linearized double-stranded DNA and 100 units Mung Bean Nuclease in 100 μ l of solution containing 30mM sodium acetate (pH 4.6), 50mM NaCl, 1mM ZnCl₂ and 5% (v/v) glycerol. Incubate at 37°C for 10 minutes (3).

C. Hairpin Loop Cleavage (cDNA)

The following can be used as the standard procedure after RNase H-mediated second strand synthesis. Combine and mix 20 μ l double-stranded cDNA (0.5–2.0 μ g), 20 μ l 5X buffer (150mM sodium acetate (pH 5.0), 250mM NaCl, 5mM ZnCl₂, 25% (v/v) glycerol), 10 units Mung Bean Nuclease, and water to a final volume of 100 μ l. Incubate at 37°C for 15 minutes. Neutralize by adding 10 μ l of 1M Tris-HCl (pH 8.0). Extract twice with buffered phenol, and precipitate with 0.5 volume 7.5M ammonium acetate and 2 volumes ethanol (6).

III. Additional Information

Molecular Weight: 39,000Da.

Requirement: Zn²⁺.

Inhibitors: Mung Bean Nuclease is inhibited by high salt concentrations (80–90% inhibition in 200–400mM NaCl). Use with 0.001% Triton® X-100 when using very low concentrations (less than 50units/ml), because under such conditions Mung Bean Nuclease may adhere to surfaces and is rather unstable (7).

IV. References

1. Ardelt, W. and Laskowski, M., Sr. (1971) Mung bean nuclease I. IV. An improved method of preparation. *Biochem. Biophys. Res. Comm.* **44**, 1205–11.
2. Kroeker, W.D., Kowalski, D. and Laskowski, M., Sr. (1976) Mung bean nuclease I. Terminally directed hydrolysis of native DNA. *Biochemistry* **15**, 4463–7.
3. Kroeker, W. and Spurgeon, S. (1985) Mung bean nuclease. *Promega Notes* **2**.
4. Mathis, D.J. *et al.* (1981) Specific in vitro initiation of transcription on the adenovirus type 2 early and late E11 transcription units. *Proc. Natl. Acad. Sci. USA* **78**, 7383–7.
5. Green, M.R. and Roeder, R.G. (1980) Definition of a novel promoter for the major adenovirus-associated virus mRNA. *Cell* **22**, 231–42.
6. Gubler, U. (1987) Second-strand cDNA synthesis: mRNA fragments as primers. *Meth. Enzymol.* **152**, 330–5.
7. Perbal, B. (1988) *A Practical Guide to Molecular Cloning*, 2nd ed., John Wiley and Sons, New York.