Certificate of Analysis

Part# 9PIM431

Revised 4/18

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 $ZnCl_2$.

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 $ZnCl_2$, 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.

AF9PIM431 0418M431

Quality Control Assays

Contaminant Assay

Endonuclease/Nickase Activity: To confirm the absence of contaminating endonuclease/nickase activity, 1µg of lambda DNA/*Hin*d 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.

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

Signed by:

R. Wheeler, Quality Assurance

Part# 9PIM431
Printed in USA. Revised 4/18

Usage Information

1. 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.

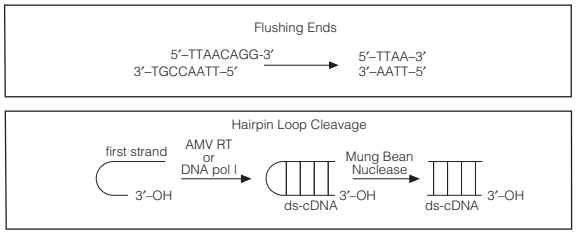

Figure 1. Mung Bean Nuclease catalyzes the generation of blunt-ended DNA and also cleaves hairpin loops to separate cDNA strands.

2. 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/µg 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 20µg linearized double-stranded DNA and 100 units Mung Bean Nuclease in 100µl of solution containing 30mM sodium acetate (pH 4.6), 50mM NaCl, 1mM $ZnCl_2$ 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 20µl double-stranded cDNA (0.5–2.0µg), 20µl 5X buffer (150mM sodium acetate (pH 5.0), 250mM NaCl, 5mM $ZnCl_2$, 25% (v/v) glycerol), 10 units Mung Bean Nuclease, and water to a final volume of 100µl. Incubate at 37°C for 15 minutes. Neutralize by adding 10µl 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).

3. Additional Information

Molecular Weight: 39,000Da.

Requirement: Zn^{2+}.

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).

4. 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 EII 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.

Part# 9PIM431
Printed in USA. Revised 4/18.