# 5´ Deadenylase

1-800-632-7799
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M0331S 001151017101

# M0331S

RR NEB2 Yes

**1,000 units** **20,000 U/ml** **Lot: 0011510**

**RECOMBINANT** **Store at –20°C** **Exp: 10/17**

**Description:** Yeast 5´ Deadenylase is a member of the HIT (histidine triad) family proteins and specifically a member of the Hint branch. It is the yeast orthologue of aprataxin. Mutations in human aprataxin have been known to be involved in the neurological disorder known as ataxia oculomotor apraxia-1 (1). The human protein has been shown to resolve abortive ligation intermediates by removing the AMP at the 5´ end of DNA (AMP-DNA hydrolase activity) (2). It also repairs DNA damage at 3´ ends by removing 3´-phosphate and 3´-phosphoglycolate (3). Human aprataxin acts on small molecules as well, nucleotide polyphosphates such as diadenosine tetraphosphate (AppppA) as well as lysyl-AMP.

The 5´ Deadenylase is encoded by *HNT3* gene of *S. cerevisiae*. NEB has shown this protein is capable of deadenylation from 5´ end of DNA and RNA, leaving the phosphate at 5´ end. It also cleaves AppppA into ATP and AMP. Its activity on lysyl-AMP is not detectable.

**Source:** Purified from an *E. coli* strain carrying a plasmid encoding 5´ Deadenylase from *S. cerevisiae*

Supplied in: 500 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 2 mM dithiothreitol, 0.1% Triton X-100 and 50% glycerol.

**Applications:**

- Deadenylation of 5´ end of DNA and RNA
- Aprataxin-dependent DNA repair assay
- Analysis of dinucleoside tetraphosphate

**Reagents Supplied with Enzyme:**
10X NEBuffer 2.

**Reaction Conditions:** 1X NEBuffer 2, 5–50 pmol adenylated DNA (AMP-DNA) in 20 µl.
**Incubate at 30°C.**

**1X NEBuffer 2:**
50 mM NaCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.9 @ 25°C

**Unit Definition:** One unit is defined as the amount enzyme required to remove 10 pmoles of AMP from a 5´-adenylated DNA oligo in 10 minutes at 30°C.

**Protocol for a Typical Deadenylation Reaction:**
Combine the following components in sterile microfuge tube:

| | |
|---|---|
| 10X NEBuffer 2 | 2 µl |
| 5–50 pmol 5´-adenylated DNA or RNA | variable |
| $H_2O$ | variable |
| 5´ Deadenylase (20 units/µl) | 1 µl |
| Total reaction volume | 20 µl |

Incubate at 30°C for 0.5–1 hour

## Quality Control Assays

**RNase Assay:** Incubation of 20 units of 5´ Deadenylase with 40 ng of RNA transcript in 10 µl for 4 hours at 37°C resulted in no detectable degradation of RNA as determined by denaturing PAGE analysis.

**Endonuclease Activity:** Incubation of 20 units of 5´ Deadenylase with 300 ng of supercoiled plasmid in 10 µl for 4 hours at 37°C produced less than 10% nicked or linear molecules as determined by agarose gel electrophoresis.

**Exonuclease Assay:** Incubation of a 50 µl reaction containing 100 units of 5´ Deadenylase with 1 µg of a mixture of single and double-stranded [$^3$H] *E. coli* DNA ($10^5$ cpm/µg) for 4 hours at 37°C released < 0.5% of the total radioactivity.

**5´ DNA Phosphatase Assay:** Incubation of 40 units of 5´ Deadenylase with 10 pmol of 5´-pDNA oligo in 10 µl for 2 hours at 37°C resulted in no detectable dephosphorylation as determined by denaturing PAGE analysis.

*(See other side)*

CERTIFICATE OF ANALYSIS

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*(See other side)*

CERTIFICATE OF ANALYSIS

**Heat Inactivation:** 70°C for 20 minutes.

**Notes:**
5´-adenylated DNA could be dsDNA, ssDNA and nicked DNA with the adenylate residue at the 5´ terminus of the nick. Deadenylation is more efficient with dsDNA and nicked DNA.

**References:**
1) Le Ber, I. et al. (2003) *Brain,* 126, 2761.
2) Ahel, I. et al. (2006) *Nature,* 443, 713.
3) Takahashi T. et al. (2007) *Nucl. Acids Res.,* 35, 3797.




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