



NYU



Omnivore RNA Pyrophosphatase: The Key to Universal 5'-end Deprotection

A versatile enzyme capable of completely removing a wide variety of 5' caps and oligophosphates from RNA.

Technology

Researchers at NYU have identified an enzyme called Omnivore RNA pyrophosphatase that is efficient at removing all kinds of protecting groups from the 5' end of RNA (triphosphates, diphosphates, canonical m⁷Gp₃ caps, and noncanonical NAD, FAD, Np₄, N-acetylglucosamine, glucose, and galactose caps; see Chart 1) to generate a 5' monophosphate. This enzyme is effective irrespective of the 5'-terminal sequence and methylation state of the RNA and even when the RNA 5' end is base paired. The wide-ranging substrate compatibility of this all-purpose RNA pyrophosphatase makes it a superior alternative to *E. coli* RppH or mRNA Decapping Enzyme. It is invaluable for analyzing a variety of cellular and synthetic RNAs, especially when the 5'-terminal protecting group reacts poorly with other available enzymes or is unknown, more than one type of 5'-terminal protecting group is present (as is typical for cellular RNA mixtures), or the 5' end is structurally sequestered by base pairing.

Background

Cellular and synthetic RNAs typically bear a protecting group at the 5' end, such as a cap or oligophosphate. A variety of important methods for analyzing individual RNAs or entire transcriptomes require the conversion of this cap or oligophosphate to a reactive 5'-terminal monophosphate on which a ligase, 5' exonuclease, or phosphatase can act. However, no enzyme that is currently available is good at removing all types of 5' protecting groups, and the available enzymes are often inhibited by 5'-terminal base pairing.

Technology ID

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Category

Life Sciences/Research
tools/Reagents
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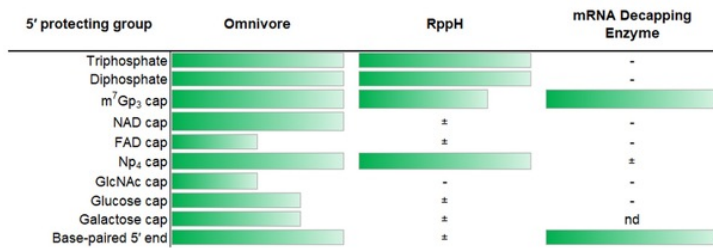


Chart 1. Comparison of the efficiency of pyrophosphatases at removing 5' protecting groups, where green bars represent complete removal of the 5' protecting group with various efficiencies indicated by bar length.

- ± Only partial removal of the 5' protecting group, even at high enzyme concentrations
- Little or no removal of the 5' protecting group, even at high enzyme concentrations
- nd Not determined
- GlcNAc *N*-acetylglucosamine

Applications

- **Transcriptome analysis:** The enzyme can be used to prepare RNA samples for transcriptome analysis by converting a wide variety of 5' protecting groups to a ligatable 5'-terminal monophosphate. This is crucial for techniques like next-generation RNA sequencing (RNA-seq), where comprehensive and accurate quantification of RNA 5' ends is often essential and the poor reactivity of some 5' ends with other RNA pyrophosphatases would be problematic.
- **RNA modification studies:** The use of Omnivore enables investigation of the functional importance of many different 5'-terminal RNA modifications in cellular processes.
- **Mapping of RNA 5' ends by RACE:** Treating RNA with Omnivore enables ligation of an oligoribonucleotide to the 5' end as a prelude to reverse transcription, PCR amplification, and 5'-terminal sequencing. Treatment with Omnivore can also be used to map RNA 5' and 3' ends simultaneously by enabling RNA circularization followed by circular RACE.
- **Synthetic RNA preparation:** When synthesizing RNA for therapeutic or research purposes, the enzyme can be used to make the 5' ends reactive by ensuring that the synthetic RNAs all have a 5'-terminal monophosphate. This is important for downstream applications such as ligation, labeling, or other enzymatic reactions that require this particular 5' end configuration.
- **Circular RNA purification:** In conjunction with XRN-1 or Terminator 5'-Phosphate-Dependent Exonuclease, Omnivore can be used to remove linear RNA contaminants from preparations of circular RNA.

Advantages

- **Broad substrate compatibility:** Applicable to RNAs bearing a wide variety of 5'-terminal protecting groups, including triphosphates, diphosphates, canonical m7Gp3 caps, and noncanonical NAD, FAD, Np₄, N-acetylglucosamine, glucose, and galactose caps.
- **Effective regardless of RNA structure:** The ability of Omnivore to deprotect structured RNA 5' ends overcomes a significant limitation of other RNA pyrophosphatases, which are often inhibited by 5'-terminal base pairing.
- **Superior performance:** Complete removal of 5'-terminal protecting groups that are difficult or impossible for other RNA pyrophosphatases to remove. See Chart 1.
- **Distinctive reliability:** Uniquely dependable when the protecting group or potential for base pairing at the 5' end is unknown or more than one type of 5'-terminal protecting group is present.

Intellectual Property

NYU has filed a provisional patent covering the method of use of Omnivore RNA pyrophosphatase.