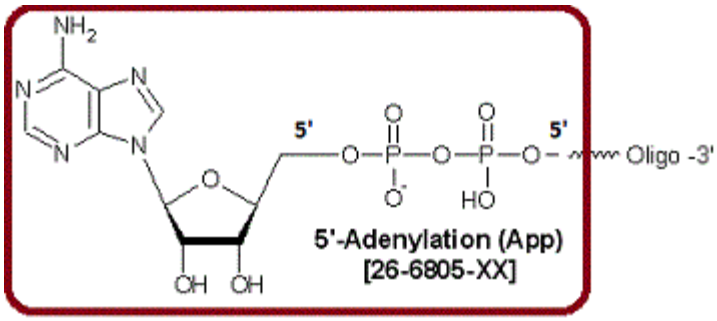
Adenylation-5' (rApp)

Category	Conjugation Chemistry
Modification Code	5rApp
Reference Catalog Number	26-6805
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	358



YIELD 5' Adenylation is performed enzymatically and thus the yield obtained is lower than other chemically modified oligos.
~400 pmole (0.4nmol) final yield for 200 nmol scale
~1 nmole final yield for 1 umol scale

The ligation of two DNA or RNA molecules, by *T4 DNA ligase* or *T4 RNA ligase*, respectively, proceeds by a mechanism in which the enzyme uses ATP to place a 5',5'-adenyl pyrophosphoryl moiety (App) onto the 5'-end of a DNA/RNA oligonucleotide. Subsequent nucleophilic attack of the pyrophosphoryl linkage of this intermediate by the 3'-OH of a second oligonucleotide produces the concatenated product, with release of AMP (1,2). The key role that 5'-adenylated DNA/RNA plays in nucleic acid ligation means that robust synthesis of stable, pre-adenylated oligonucleotides (5'-App oligos), which act as substrates for T4 ligases in the absence of ATP, could be beneficial in any experimental study involving ligation.

Gene Link provides 5'-adenylation of **any** DNA or RNA oligonucleotide synthesized by us, as a custom service. To prevent the 5'-App oligo from self-ligating, the 3'-end is capped with a 3'-terminal blocking group, such as a dideoxy nucleotide or 3'-amino linker, which lack 3'-OH groups. The resulting 5'-App oligonucleotide is stable and ready for use in any ligation-based application. Examples of such applications include the following:

(a) miRNA library construction/next-generation sequencing: miRNAs processed *in vivo* are short (21-23 nt) and have 5'-phosphate and 3'-OH termini. Consequently, construction of high-quality miRNA libraries from cellular RNA is difficult, because attempts to ligate adaptors to miRNA ends, using *T4 RNA ligase* and ATP, results in a high level of undesirable miRNA self-ligation. This problem can be eliminated by first using the ligase to attach a 5'-App-modified adaptor to the 3'-OH end of the miRNA **in the absence of ATP**, and then attaching a second adaptor to the 5'-phosphate end of the resulting miRNA-3'-adaptor molecule using the ligase in the presence of ATP. The miRNA library will now have the appropriate adaptors at both ends, and can be cloned into a suitable vector for subsequent sequencing (3-5).

(b) activated nucleic acid substrates for *in vitro*-selected ribo/deoxyribozymes: Performing detailed structure-function studies on long, catalytically-active, naturally-occurring RNA (*e.*

g., group I and II introns, ribonuclease P) often requires incorporation of site-specific modifications. However, because such modification is currently not possible by *in vitro* transcription methods, various combinations of modified, chemically synthesized RNA oligos and RNA transcripts must be ligated together to generate the desired long, modified RNA molecule. Since T4 ligases often exhibit low yields and a limited range of possible substrates when used to form such RNAs by ligation, *in vitro*-selected ribozymes/deoxyribozymes are being developed to broaden the available selection of ligation strategies (6). 5'-App oligonucleotides, containing desired site-specific modifications, can be used as activated substrates for these ribo/deoxyribozymes, both during the initial selection process used to develop them, and during the subsequent construction of a particular long RNA to be used in a structure-function study.

(c) broaden the range of explorable RNA substrates for ribo/deoxyribozymes: The RNA variant 5'-triphosphorylated RNA (5'-pppRNA) is produced by *in vitro* transcription with *T7 RNA polymerase* (7). Both natural ribozymes and *in vitro*-selected ribo/deoxyribozymes can use 5'-pppRNA as a substrate for ligation, with the 5'-leaving group being pyrophosphate (PPi). However, for a 5'-pppRNA produced *in vitro* by *T7 RNA polymerase*, the 5'-pppNTP is 5'-pppG. 5'-AppRNA is structurally similar to 5'-pppRNA, and the 5'-AMP leaving group, like PPi, is also good. However, 5'-AppRNA can be synthesized with any nucleotide at the 5'-end, not only G. Thus, substitution of 5'-AppRNA for 5'-pppRNA broadens the range of sequences that can be explored as RNA substrates for both natural and *in vitro*-selected ribozymes/deoxyribozymes.

(d) activated 5'-pyrimidine-rich RNA: RNA that is pyrimidine-rich at the 5'-end is often difficult or even impossible to transcribe *in vitro* with phage polymerases. Consequently, obtaining pyrimidine-rich RNA that is 5'-phosphorylated, and thus suitable for ligation, is a major challenge (8). 5'-adenylation of such RNA molecules could prove to be a viable strategy for activating them for ligation.

(e) 5'-end labeling: The adenylate group attached to the oligo contains a ribose 2',3'-diol moiety that can be oxidized to aldehydes using sodium periodate. Consequently, a 5'-App nucleic acid could, after such oxidation, be labeled at the 5'-end with fluorescent dyes or other biophysical probes via reductive amination of the aldehydes (3,9). This labeling strategy would be useful in cases where insertion of a particular 5'-end modification using solid phase synthesis is either not possible or not desired.

References

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