# TECHNOLOGY OFFER

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## Identification and uses of a novel mammalian RNA 2', 3'-cyclic phosphatase

We present ANGEL2, a mammalian RNA processing enzyme with the unique ability to hydrolyze RNA terminal 2',3'-cyclic phosphates and 2'-phosphates into 2',3'-OH. We propose recombinant ANGEL2 as a tool in cloning protocols for whole transcriptome shotgun sequencing (RNA-seq), to specifically uncover RNA species ending with 2'-, 3'- or 2',3'-cyclic phosphate groups. The lack of reactivity towards 3'-P distinguishes ANGEL2 from well-known, commercially available enzymes such as T4 polynucleotide kinase or alkaline phosphatase. Highly active, recombinant ANGEL2 can be efficiently produced using bacterial or insect cell expression systems.

**REFERENCE:**
675.17

**DEVELOPMENT STATUS:**
Recombinant enzyme is available

**IPR:**
EP18195389

**KEYWORDS:**
ANGEL2
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UPR

**AVAILABLE FOR:**
- R&D collaboration
- License Agreement

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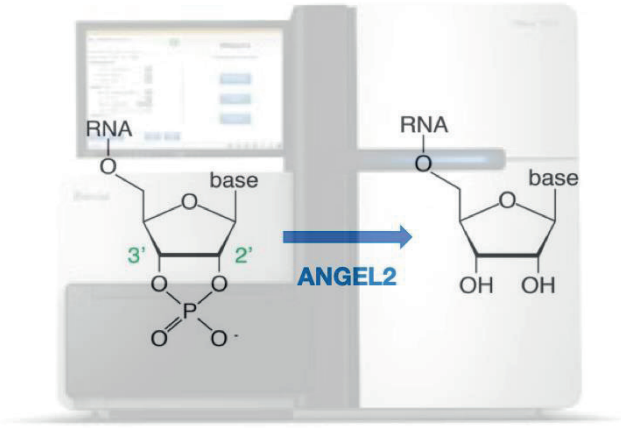
## BACKGROUND

Cellular RNA molecules can display 3'-hydroxyl (3'-OH), 3'-phosphate (3'-P) or 2',3'-cyclic phosphate (2',3'>P) termini. While 3'-OH ended RNA species are readily cloneable using standard RNA-seq library preparation protocols, RNAs ending with 2'P-, 3'-P or 2',3'>P groups require laborious and artefact-prone processing and therefore remain mostly elusive. RNA molecules with a terminal 2',3'>P arise from endonuclease cleavage, exonuclease trimming, or de novo synthesis by the RNA 3'-terminal phosphate cyclase, RTCA, acting on yet unidentified 3'-P ended RNAs. The only enzyme known so far to convert 2',3'>P into 2',3'-OH is the bacteriophage T4 polynucleotide kinase (T4 PNK). However, T4 PNK also removes 2'-P and 3'-P. In turn, Alkaline phosphatase (CIP) removes terminal 2'-P and 3'-P, but does not react towards 2',3'>P.

## TECHNOLOGY

We have identified and characterized human ANGEL2, a predicted deadenylase, as a novel, unique and highly active RNA 2',3'-cyclic phosphatase able to convert 2',3'>P and 2'-P, 3'-OH termini, but not 2'-OH, 3'-P, into 2',3'-OH. We propose the technological use of recombinant ANGEL2 in cloning protocols for RNA-seq, to identify cellular RNAs terminating in 2'P or 3'-P (main use), or 2',3'>P (minor use). Recombinant ANGEL2 can be supplied alone or as part of a kit, with 2',3'>P and 2',3'-OH RNA substrates as positive and negative controls, respectively, and with appropriate buffers (pH 7-9) and divalent metal ions such as $Mg^{2+}$, $Mn^{2+}$, $Ca^{2+}$ in a final concentration range of 2-50 mM.

Figure: The peculiar substrate specificity of ANGEL2 makes it a unique reagent for cloning protocols to identify RNAs of the hidden transcriptome layer.




| Cellular RNA substrates | RNA products after treatment with | | |
|---|---|---|---|
| | CIP | T4 PNK | ANGEL2 |
| 3′-OH | 3′-OH | 3′-OH | 3′-OH |
| 3′-P | 3′-OH | 3′-OH | 3′-P |
| 2′-P | 3′-OH | 3′-OH | 3′-OH |
| 2′,3′>P | 2′,3′>P | 3′-OH | 3′-OH |

## BENEFITS

- ANGEL2 exclusively reacts towards RNAs containing 2',3'>P and 2'-P.
- ANGEL2 is not reactive towards 3'-P ended RNAs (CIP and T4 PNK activities).
- ANGEL2 does not dephosphorylate 5'-P termini (CIP activity).
- ANGEL2 substrates includes single and double stranded RNAs.
- ANGEL2 can be produced with high yields using bacterial and insect cell expression systems.

## ADVANTAGE

ANGEL2 as a reagent in cloning protocols for RNA-seq analysis to identify novel RNA species that have so far escaped detection using commercially available reagents such as CIP and T4 PNK.

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