CELLSCRIPT™
RNA for Translation in Cells

Adenylator™ DNA/RNA 5'-Adenylation Kit

Cat. No. **ADR250610**

INTRODUCTION

The Adenylator™ DNA/RNA 5'-Adenylation Kit enables efficient 5′-end adenylation of 5'-monophosphorylated DNA or RNA oligonucleotides, providing a powerful, catalytic, low-temperature alternative to 5′-end adenylation methods that use traditional thermostable RNA ligases. Optimized for use in next-generation sequencing workflows[1] (e.g., small RNA library prep and Native elongating transcript sequencing [NET-seq][2,3]) and miRNA library construction,[4] the Adenylator Enzyme converts 5′-monophosphorylated oligos into 5'-adenylated substrates compatible with T4 RNA Ligase 2 truncated or truncated/K227Q-based reactions. Unlike Mth RNA Ligase, which relies on high temperature to minimize oligo circularization and concatemerization events, Adenylator Enzyme performs robustly at 37°C, acts catalytically and cannot catalyze oligo self-ligation (see Figures 3A & 3B). The Adenylator DNA/RNA 5'-Adenylation Kit offers a cost-effective, scalable solution for labs generating adenylated oligos from monophosphorylated DNA/RNA oligos. Each 10-reaction kit will adenylate 3 nmoles of monophosphorylated DNA/RNA oligos.

Applications:

- Next-Generation Sequencing (NGS): Efficiently generates adenylated DNA linkers for downstream use in generating RNA libraries and NGS applications.

Benefits:

- Higher turnover rate: Adenylator Enzyme has a high specificity and turnover rate for p-DNA and p-RNA 5' ends to efficiently generate 5' AppDNA and 5' AppRNA.
- No ligase activity: No off targets effects to generate unwanted side products due to concatemerization or circulation of p-DNA or p-RNA oligos.
- Single step reaction
- Cost-effective alternative to purchasing chemically-synthesized adenylated oligos.

726 Post Road, Madison, WI 53713 • 888-826-5586 (U.S. only) • (608) 442-6484 • Fax (608) 442-6187

MATERIALS

Materials Supplied

Important Store at –20°C in a freezer without a defrost cycle. Do not store at –70°C.

Adenylator™ DNA/RNA 5'-Adenylation Kit Contents (10 reactions)	
Kit Component	Volume
Adenylator™ Enzyme in 50% glycerol, 50 mM Tris-HCl, pH 7.5, 0.25 M NaCl, 1 mM dithiothreitol (DTT), 0.1 mM EDTA and 0.1% Triton® X-100.	20 µl
10X Adenylation Reaction Buffer	20 µl
10 mM ATP	20 µl
50 µM Control Phosphorylated Oligo	12 µl
RNase-Free Water	500 µl

Materials Required, but not Supplied

- 5'-monophosphorylated DNA or RNA oligonucleotide (100 µM)
- Isopropanol or ethanol (for salt/alcohol precipitation purification of the adenylated reaction product)
- 3 M NaOAc (pH 5.2), 5 M NaCl, 5 M LiCl or 5 M NH_4OAc (for salt/alcohol precipitation purification of the adenylated reaction product)
- 70% Ethanol
- Optional: Colored nucleic acid coprecipitant (e.g., Glycoblue™ Coprecipitant [Thermo Fisher] or Pellet Paint® [Sigma Millipore])
- Optional: Materials for polyacrylamide gel electrophoresis.
- Optional: ScriptGuard™ RNase Inhibitor (sold separately) if adenylating RNA oligos.

SPECIFICATIONS

Functional Testing

One Adenylator DNA/RNA 5'-Adenylation Kit reaction 5' adenylates 300 pmoles of oligonucleotide in 60 minutes at 37°C under standard reaction conditions.

The Adenylator DNA/RNA 5'-Adenylation Kit is functionally tested by performance of the kit control reaction. The reaction product is then treated with a 5'-monophosphate-dependent 5'→3' exoribonuclease (XRN1) with reaction product visualization via PAGE.

Control Phosphorylated Oligo

The Control Phosphorylated Oligo supplied in the kit is a 5'-monophosphate-containing 23-b DNA oligonucleotide.

Contaminating Activity Assays

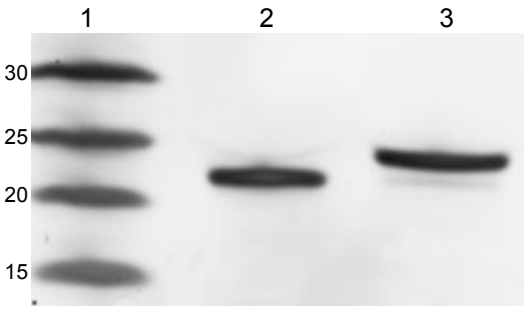
All components of the Adenylator DNA/RNA 5'-Adenylation Kit are free of detectable RNase and DNase activity.

For more information, consult the appropriate safety data sheet (SDS) at www.cellscript.com.

EXAMPLE DATA

♦ **Figure 1. Adenylation of a 23-nt 5'-monophosphate DNA Oligo using: Adenylator Enzyme.**

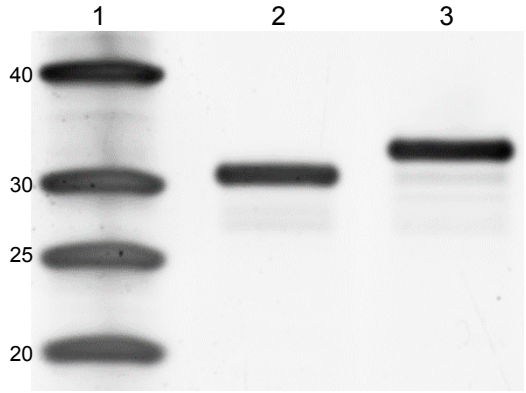
20% acrylamide, 8 M Urea, 1X TBE gel stained with SYBR®-Gold



1) IDT ssDNA 10/60 Ladder
2) Untreated (unadenylated) p-DNA Oligo (23-nt)
3) Treated (adenylated) p-DNA Oligo (24-nt)

♦ **Figure 2. Adenylation of a 26-nt 5'-monophosphate RNA Oligo using: Adenylator Enzyme.**

20% acrylamide, 8 M Urea, 1X TBE gel stained with SYBR-Gold

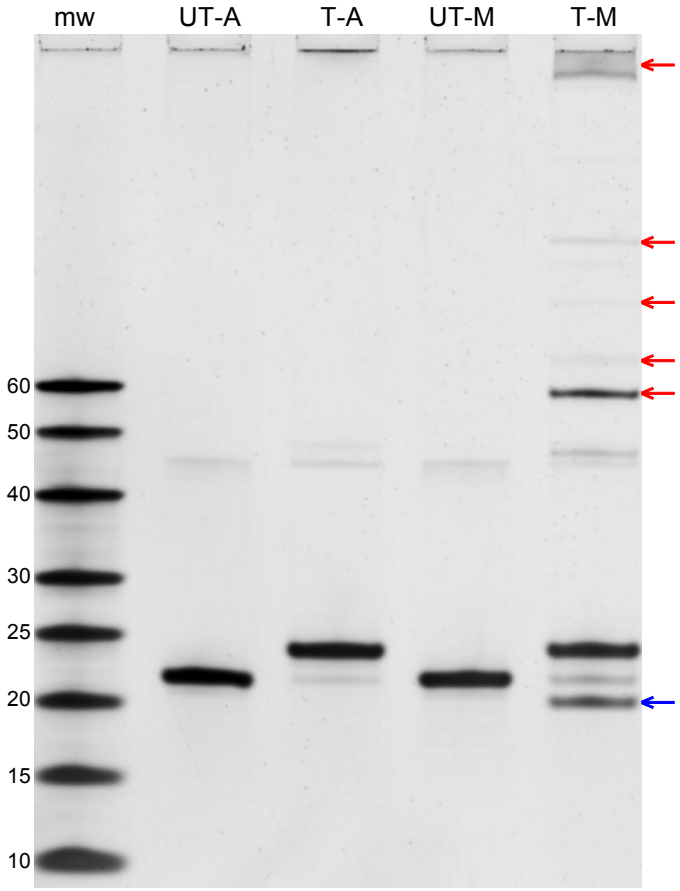


1) IDT ssDNA 10/60 Ladder
2) Untreated (unadenylated) p-RNA Oligo (26-nt)
3) Treated (adenylated) p-RNA Oligo (27-nt)

♦ **Figures 3A Comparative adenylation of a 5'-monophosphate DNA Oligo using: Adenylator Enzyme and Mth RNA Ligase.**

- Adenylator treatment cannot form substrate concatemers.
- Adenylator treatment cannot circularize oligo substrates.

20% acrylamide, 8 M Urea, 1X TBE gel stained with SYBR-Gold gel



mw) IDT ssDNA 10/60 Ladder

UT-A) Untreated (unadenylated) p-DNA Oligo (23-nt) in Adenylator reaction mix.

T-A) Adenylator Enzyme Treated (37°C) p-DNA Oligo (24-nt).

UT-M) Untreated (unadenylated) p-DNA Oligo (23-nt) in Mth RNA Ligase reaction mix.

T-M) Mth RNA Ligase Treated (65°C) p-DNA Oligo (24-nt).

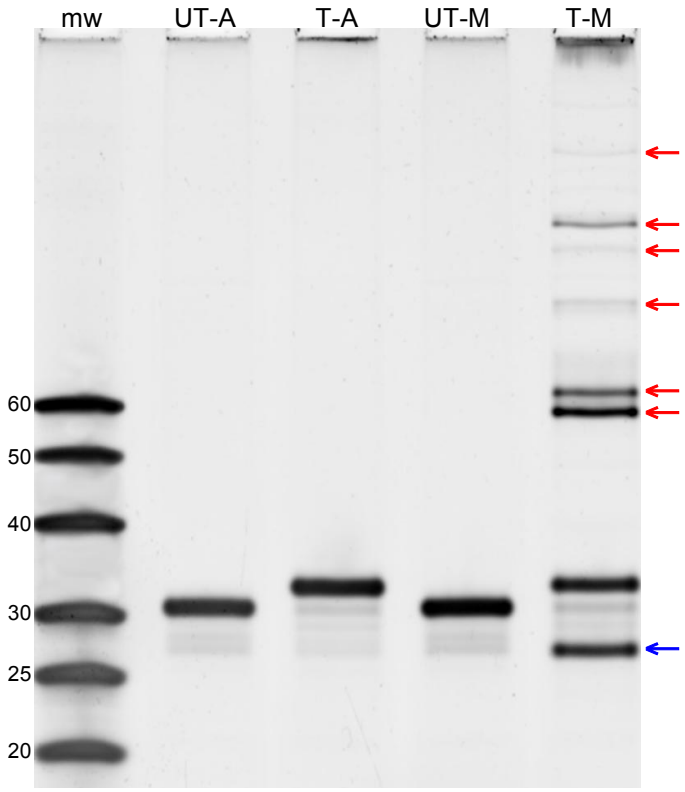
←) DNA Oligo Concatemers

←) Circular DNA Oligo

♦ **Figures 3B Comparative adenylation of a 5'-monophosphate RNA Oligo using: Adenylator Enzyme and Mth RNA Ligase.**

- Adenylator treatment cannot form substrate concatemers.
- Adenylator treatment cannot circularize oligo substrates.

20% acrylamide, 8 M Urea, 1X TBE gel stained with SYBR-Gold gel



mw) IDT ssDNA 10/60 Ladder

UT-A) Untreated (unadenylated) p-RNA Oligo (26-nt) in Adenylator reaction mix.

T-A) Adenylator Enzyme Treated (37°C) p-RNA Oligo (27-nt).

UT-M) Untreated (unadenylated) p-RNA Oligo (26-nt) in Mth RNA Ligase reaction mix.

T-M) Mth RNA Ligase Treated (65°C) p-RNA Oligo (27-nt).

←) RNA Oligo Concatamers

←) Circular RNA Oligo

PROCEDURE

A. Synthesis of Adenylated Oligonucleotides

1. The standard reaction will adenylate 300 pmoles of 5'-monophosphorylated DNA or RNA oligo.

   Thaw the reagents, then place on ice.

   Combine the following reagents in the order indicated below:

Adenylator DNA/RNA 5'-Adenylation Reaction	
Component	Amount
RNase-Free Water	11 µl
10X Adenylation Reaction Buffer	2 µl
10 mM ATP	2 µl
5'-monophosphorylated DNA or RNA oligo (100 µM, 100 pmol/µl)	3 µl
Adenylator Enzyme	2 µl
Total Volume	20 µl

Important If performing the kit control reaction, use 6 µl of 50 µM Control Phosphorylated Oligo and 8 µl of RNase-Free Water.

If adenylating RNA oligos, we recommend the inclusion of ScriptGuard RNase Inhibitor (1 U/µl final reaction volume, sold separately) in the reaction.

The reaction can be scaled up as desired.

2. Mix gently and incubate at 37°C for 1 hour.

3. Stop the reaction using any one of the following methods:
   a) Proceed immediately to Adenylated Oligo Recovery
   b) Immediate storage at –20°C or –70°C.
   c) Addition of EDTA to a final concentration of > 11 mM.
   d) Heat inactivate by incubation at 70°C for 10 minutes.

B. Adenylated Oligo Recovery

This purification method describes NaOAc/Isopropanol precipitation, alternative purification methods may be used.

I) **NaOAc / Isopropanol Precipitation**:

Note: this purification method does not remove proteins or unincorporated ATP.

1) Add one-tenth volume (2 µl) 3 M NaOAc (pH 5.2) and three volumes (60 µl) isopropanol to the reaction tube. Optional: a colored coprecipitant may also be included in order to visualize the pellet.
2) Mix well then incubate for 30 minutes at –20°C or 15 minutes at –70°C.
3) Pellet the adenylated oligo by centrifugation at >10,000 x g for 15 minutes at 4°C.
   Note: the pellet may not be visible.
4) Carefully remove the supernatant with a pipette without disturbing the pellet and discard. Gently rinse the pellet with 200 µl 70% ethanol.
5) Pellet the rinsed adenylated oligo by centrifugation at >10,000 x g for 5 minutes at 4°C.
6) Carefully remove the 70% ethanol with a pipette without disturbing the pellet and discard.
7) Allow the pellet to air dry for 5-10 minutes, then resuspend in the desired volume of RNase-Free Water (e.g., 10-20 µl).

II) **Alternative Salt / Alcohol Precipitations**:

Note: these purification methods do not remove proteins or unincorporated ATP.

Any of the following can be substituted into the purification method described above in method I.

1) one-tenth volume (2 µl) 5 M NaCl in place of NaOAc.
2) one-tenth volume (2 µl) 5 M LiCl in place of NaOAc.
3) one-tenth volume (2 µl) 5 M NH_4OAc in place of NaOAc.
4) three volumes (60 µl) ethanol in place of isopropanol.

C. Visualization of the Adenylated Oligo (optional)

For purposes of assaying reaction efficiency, if desired, the final reaction product adenylated oligo can be run against the unadenylated, 5'-monophosphorylated oligo starting material. Load 10-20 pmol of each onto a 20% polyacrylamide, 8 M Urea gel. See example in Figs. 1 & 2, page 3.

1. While the reactions are incubating, prepare a 20% acrylamide/8 M Urea/1X TBE gel.
   a) Blow out unpolymerized acrylamide from the wells immediately after comb removal.
   b) Pre-run the gel at 300 volts constant voltage for 20-30 minutes.
   c) Blow out the excess urea from the wells just prior to sample loading.
2. Load 5 µl (10-20 pmoles) of each stopped reaction mix to the appropriate lanes for PAGE.
   a) Load the treated sample adjacent to the untreated control sample.
   b) Running a molecular weight marker, while not necessary for analysis, is optional.
3. Run the gel at 300 volts (constant voltage) for 1 hours.
4. Stain the gel with a dye appropriate for your gel visualization system (e.g., SYBR Gold Nucleic Acid Gel Stain [Invitrogen]).
5. Visualize/image the gel.

TROUBLESHOOTING

Symptom	Solution
Lack of 5' Adenylation	Check adenylation reaction efficiency by polyacrylamide gel electrophoresis (20% PA, 8 M Urea) against the unadenylated, 5'-monophosphorylated oligonucleotide starting material. See potential reasons below.
	ATP solution is degraded. Repeat the reaction using a fresh tube of ATP.
	Oligonucleotide starting product is not 5'-monophosphorylated. Verify the oligo.
	Substrate oligonucleotide starting product contains stable secondary structure on the 5' end preventing access of the 5' phosphate to the Adenylator Enzyme. Verify the predicted 5' end secondary structure of the substrate oligo.
	Adenylator Enzyme is inactivated. Contact CELLSCRIPT Technical Services.
RNA Oligo is Degraded	Be sure to include ScriptGuard RNase Inhibitor in the reaction. Take all common RNase contamination preventative steps (e.g., gloves, RNase-Free tips and tubes, etc.).

RELATED PRODUCTS

– ScriptGuard™ RNase Inhibitor

REFERENCES

1. Vigneault F. et al. (2008) Nature Methods 5, 777.
2. Churchman L.S. and Weissman J.S. (2011) Nature 469, 7330. doi: 10.1038/nature09652.
3. Churchman L.S. and Weissman J.S. (2012) Curr. Protoc. Mol. Biol. Apr:Chapter 4:Unit 4.14.1-17. doi: 10.1002/0471142727.mb0414s98.
4. Pak J., Fire A. (2007) Science 315, 241.

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