5' DNA Adenylation Kit

Product description

5' DNA Adenylation Kit is specifically designed for the adenylation modification of 5'-phosphorylated single-stranded DNA (ssDNA) at the 5' end. This kit contains Mth RNA Ligase (an RNA ligase derived from thermophilic archaea) and ATP. During the adenylation reaction, 5'-phosphorylated single-stranded DNA (5'-pDNA) serves as the substrate. The Mth RNA Ligase, which possesses adenylation activity, hydrolyzes ATP into AMP and PPi, and subsequently transfers the AMP to the 5'-phosphate group of the ssDNA, ultimately generating 5'-adenylated ssDNA (5'-AppDNA).

This kit offers a simple and efficient one-step reaction for quantitative adenylation, typically converting 95% of pDNA to AppDNA, eliminating the need for product purification. It is more convenient than current chemical or enzymatic methods. The reaction temperature of 65°C effectively reduces secondary structure formation, and is suitable for reaction systems ranging from pmol to μmol substrate amounts. This product is commonly used to generate adenylated linkers for 3'-end ligation to ssRNA/ssDNA with a 3'-hydroxyl group, facilitating applications such as cloning, high-throughput sequencing library preparation, or PCR detection.

Composition and storage conditions

Components \ Size	10 rxns	50 rxns	Storage
Mth RNA Ligase	20 μL	100 μL	-20°C
10× Adenylation Reaction Buffer	100 μL	500 μL	-20°C
ATP (1 mM)	25 μL	125 μL	-20°C
Shipping: Dry Ice	**Shelf life:** 12 months		

Experimental operation

Adenylation of the 5' end of phosphorylated ssDNA/ssRNA:

1. Refer to the table below to prepare the reaction system on ice:

Reagent	20 μL Reaction	Final Concentration
10× Adenylation Reaction Buffer	2 μL	1×

Phosphorylated ssDNA or ssRNA	X μL	5 μM
ATP (1 mM)	2 μL	0.1 mM
Mth RNA Ligase	2 μL	
Nuclease-free Water	to 20 μL	

***Note:**

a. The adenylylation reaction system can be scaled up according to the proportions in the table above, with no significant effect on the yield of the adenylylated product.

b. If RNA manipulation is involved, strict adherence to RNA handling protocols is required to avoid RNase contamination. Relevant reagents and consumables should be DEPC-treated to remove RNase or be confirmed as RNase-free. If ssRNA is involved, it is recommended to add an appropriate amount of RNase Inhibitor (Cat. No. K1046).

2. Incubate at 65℃ for 60 min.

***Note:** In some cases, to make the connection reaction more thoroughly, the reaction time can be appropriately extended.

3. Incubate at 85℃ for 5 min to terminate the reaction.

4. Perform electrophoretic identification of adenylylated products. Before loading the samples for electrophoresis, incubate them at 95℃ for 5 min and then place on ice to ensure complete denaturation. It is recommended to use a 15% denatured polyacrylamide gel containing 7 M urea for gel electrophoresis analysis.

***Note:** The molecular weight of the sample will increase after adenosylation modification.

5. The sample can subsequently be concentrated using the conventional ethanol precipitation method, and then subjected to 3'-hydroxyl ligation with small RNAs using enzymes such as T4 RNA Ligase 2.

Notes

1. The substrate required for adenylylation must be ssDNA/ssRNA with a 5' phosphate group. The 3' end can be aminated or otherwise blocked, or it can remain unblocked.

2. The optimal reaction temperature for the enzyme included in the kit is 65°C. De-adenylylation may occur at 25°C. Therefore, it is recommended to heat the reaction at 85°C for 5 min after completion to inactivate the enzyme. If the enzyme is not inactivated, exposure to room temperature conditions in subsequent steps may lead to a decrease in the adenylylation ratio.

3. This product is for scientific use only!

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com