Manual

Version 1.2

Product name: 5′ Adenylation Kit

Cat #: APPK-100, APPK-200

Description:

5´ DNA Adenylation Kit is for the enzymatic synthesis of 5′ adenylated ssDNA linkers. The kit is optimized to produce the adenylated DNA with or without 3´ terminator. The 5´ DNA adenylation kit can simply and efficiently generate greater than 95% conversion of pDNA to AppDNA.

Features:

- One step reaction. Simple and efficient.
- 65°C reaction temperature reduces secondary structural concerns.
- Highly efficient process eliminates the need for purification of the product.
- Easily scale up from pmol to µmol range.

Application:

Enzymatic 5´adenylation of single-stranded DNA linkers for next generation sequencing (NGS).

Kit Components:

- 10X 5′ DNA Adenylation Reaction Buffer
- 1 mM ATP
- Mth RNA Ligase

Recommended Storage Condition: -20 °C

Protocol:

1. Remove all supplied reagents from -20°C storage and thaw on ice.
2. Briefly centrifuge thawed reagents, then keep on ice.
3. Set up the 5′ adenylation reaction in a PCR microtube:

Reagent	Volume (µl)
5′ phosphorylated DNA oligonucleotides	x (around 200 pmol)
10x 5′ Adenylation Buffer	2
1 mM ATP	2
Mth RNA ligase	2
Nuclease-free water	14-x
Total volume	20

4. Gently pipette the mixture up and down several times to mix thoroughly, then briefly centrifuge to collect the liquid to the bottom.
5. Place the PCR microtube in a thermo cycler with the lid heated at 100 °C and run the following program:

   60 min @ 65 °C

   10 min @ 85°C

   Keep @ 4 °C

6. Remove the sample from the thermo cycler and keep on ice until further analysis or purification.

Note:

1. The adenylated products can be used as 3′ end RNA sequencing adapters. However, the starting 5′ phosphorylated DNA oligos need to be blocked at their 3′ end (3′ dideoxycytidine (ddC) modification **or** 3′ C3 spacer) in order to prevent self-ligation in the presence of RNA ligase 2, truncated.
2. The provided reaction recipe can be scaled up without compromising the yield.