



Preparation of the adenylated oligonucleotide for mapping 3' OH RNA termini

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ABSTRACT

Adapted from the protocol for oligonucleotide 5' adenylation (E2610) of *New England Biolabs* (NEB).

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME

5' DNA Adenylation Kit - 10 rxns111212

CATALOG

E2610S

VENDOR



New England
Biolabs

1 Mix the following components (20 μ L):

Component	Amount [μ L]	Final concentration
5' phosphorylated DNA oligo [50 μ M]	4	10 μ M
10 \times 5' DNA Adenylation buffer	2	1 \times
ATP [1 mM]	2	0.1 mM
Mth RNA ligase [50 pmol/ μ L]	4	10 μ M
RNase-free water (ddH ₂ O)	8	

2 Incubate for 60 minutes at 65 °C.

3 Incubate for 5 minutes at 85 °C to inactivate the enzyme.

4 The adenylated oligo can be directly used for RNA ligation with T4 RNA ligase 2 (optimally with T4 RNA Ligase 2, truncated KQ (NEB #M0373)).



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