

SI #2 5' terminal phosphorylation of RNA oligomers

The standard procedure involves phosphorylation by kinase and [γ - ^{32}P] ATP. Phosphorylation was carried out by adding 1 μL of T4 Polynucleotide kinase PNK (EC 2.7.1.78, 10 U/ μL , New England Biolabs) (Ipswich, MA, USA; # M0201L), 2 μL of 10 \times PNK buffer and 0.5 μL [γ - ^{32}P]ATP to the neo-polymerized RNA in 20 μL , followed by incubation at 37 $^{\circ}\text{C}$ for 30 min. This enzyme catalyzes the transfer and exchange of P_i from the γ -position of ATP to the 5'-OH terminus of polynucleotides, and the removal of 3'-phosphoryl group from 3'-phosphoryl polynucleotides. One unit is defined as the amount of enzyme catalyzing the production of 1 nmol of phosphate to the 5'-OH end of an oligonucleotide from [γ - ^{32}P] ATP in 30 min at 37 $^{\circ}\text{C}$. This procedure typically provides a specific activity of 15000 cpm/pmol.