

Protocol for DNA/RNA 5'-end labeling by T4 PNK in the forward reaction

1. Prepare the following reaction mixture:

Dephosphorylated DNA or Oligonucleotide	1-20 pmol of 5'-termini 10-50 pmol
10X reaction buffer A	2 μ L
$[\gamma\text{-}^{32}\text{P}$ or $\gamma\text{-}^{33}\text{P}]$ -ATP	20 pmol
T4 Polynucleotide Kinase	1 μ L (10 U)
Water, nuclease-free (#R0581)	to 20 μ L
Total volume	20 μ L

2. Incubate at 37 °C for 30 min.

3. Add 1 μ L 0.5 M EDTA (pH 8.0) and incubate at 75 °C for 10 min.

4. Separate labeled DNA from unincorporated label by gel filtration on Sephadex G-50.

Protocol for DNA 5'-end labeling by T4 PNK in the exchange reaction

1. Prepare the following reaction mixture:

Linear DNA	1-20 pmol of 5'-termini
10X reaction buffer B	2 μ L
$[\gamma\text{-}^{32}\text{P}$ or $\gamma\text{-}^{33}\text{P}]$ -ATP	40 pmol
24% (w/v) PEG 6000 solution	4 μ L
T4 Polynucleotide Kinase	1 μ L (10 U)
Water, nuclease-free (#R0581)	to 20 μ L
Total volume	20 μ L

2. Incubate at 37 °C for 30 min.

3. Add 1 μ L 0.5M EDTA (pH 8.0) and incubate at 75 °C for 10 min.

4. Separate labeled DNA from unincorporated label by gel filtration on Sephadex G-50.

Note

- If ethanol solution of $[\gamma\text{-}^{32}\text{P}$ or $\gamma\text{-}^{33}\text{P}]$ -ATP is used, dry the required amount of ATP under vacuum and dissolve in water, nuclease-free.
- The ATP concentration should be at least 1 μ M in the forward reaction and at least 2 μ M in the exchange reaction (3, 4).

Protocol for Phosphorylation of DNA

1. Prepare the following reaction mixture:

Linear ds DNA or Oligonucleotide	1-20 pmol of 5'-termini 10-50 pmol
10X reaction buffer A for T4 Polynucleotide Kinase	2 μ L
ATP, 10 mM*	2 μ L
T4 Polynucleotide Kinase	1 μ L (10 U)
Water, nuclease-free (#R0581)	to 20 μ L
Total volume	20 μ L

* Prepare 10 mM ATP solution by combining 10 μ L of 100 mM ATP solution (#R0441) and 90 μ L of Water, nuclease-free.

2. Mix thoroughly, spin briefly and incubate at 37 °C for 20 min.

3. Heat at 75 °C for 10 min.

Note

Visit www.thermofisher.com for molar calculations.