TechTips

TECH TIP 205

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USB® OptiKinase™ Eliminates Base Bias in Labeling of Oligonucleotide 5'-Ends

Wild-type T4 Polynucleotide Kinase (PNK)[1,2,3] is commonly used for phosphorylation and labeling of 5'-ends of DNA and oligonucleotides. Labeling, in particular, can be accomplished by two approaches, the forward reaction and the exchange reaction (Fig. 1)[3]. Historically, T4 PNK has played a crucial role in molecular biology and it continues to be important in applications such as labeling of probes for hybridization, sequencing or mapping of transcripts and in phosphorylation of DNA ends for cloning[3,4]. Although T4 PNK continues to be widely used, the enzyme exhibits two limitations. First, T4 PNK exhibits base bias; the effectiveness of phosphorylation depends on the base at the 5'-end of the oligonucleotide target, with 5'-C exhibiting the lowest extent of phosphorylation[5]. Second, T4 PNK can be challenging to use; precise enzyme titrations and careful optimization of reaction times are often required in order to achieve desired results[3,6]. USB OptiKinase™ overcomes these limitations, greatly simplifying and improving phosphorylation and labeling reactions.

OptiKinase is a recombinant version of T4 PNK that has been genetically modified to accomplish uniform and consistent phosphorylation and labeling of 5'-ends of DNA and oligonucleotides. Base bias is dramatically reduced, which should greatly increase uniformity of labeling of diverse templates. The need for careful optimization of reaction conditions is also reduced, which should improve consistency of results between experiments. Finally, lower amounts of radioactive ATP can be used without sacrificing labeling efficiency, resulting in reduced reagent costs. Thus, USB OptiKinase offers substantial advantages over wild-type T4 PNK.

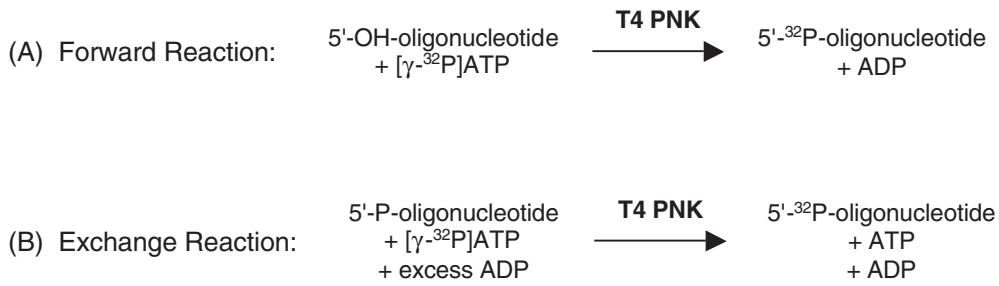

Fig. 1. Labeling of oligonucleotides with T4 PNK by the forward reaction and the exchange reaction. (A) In the forward reaction, T4 PNK catalyzes the transfer of the radiolabeled γ-phosphate group of ATP to the free 5'-OH group of the oligonucleotide, yielding labeled phosphorylated oligonucleotide and ADP. (B) In the exchange reaction, in the presence of excess ADP and a specific buffer, T4 PNK catalyzes the exchange of the radiolabeled γ-phosphate group of ATP with the non-labeled 5'-phosphate group of the oligonucleotide, yielding labeled phosphorylated oligonucleotide and non-labeled ATP.

Method

The following protocol corresponds to use of OptiKinase for labeling of oligonucleotides by the forward reaction. The protocol is similar to that for T4 PNK, with the exception that there is no need to use excess radiolabeled ATP.

1. Combine the following:

Oligonucleotide (5 pmol)	__	µl
OptiKinase Buffer (10X)	2.5	µl
[γ-^{32}P]ATP* (5 pmol)	__	µl
Water	__	µl
OptiKinase	10	units
Total	25	µl

2. Mix the contents well and centrifuge briefly. Incubate at 37°C for 30 min.
3. Terminate the reaction by heating at 65°C for 10 min.

   Optional performance assay: The amount of radioactive phosphate incorporated into 5'-ends may be determined by separating 5'-end-labeled oligonucleotide from precursor ATP by binding to DE81 filter paper and washing the filter with a solution of 5% Sodium Phosphate dibasic.

* Either [γ-^{32}P]ATP or [γ-^{33}P]ATP (≥ 3000 Ci/mmol) may be used. Whether OptiKinase or T4 PNK is used, best results are obtained by using very fresh radiolabeled ATP. Even a few days (less than one half life) can dramatically decrease the specific activity of the final product.

Results

OptiKinase offers a variety of advantages over T4 PNK. Primary among these is that OptiKinase overcomes base bias, allowing uniform labeling of oligonucleotides (Fig. 2). Additionally, OptiKinase shifts the reaction equilibrium toward phosphorylation, allowing efficient labeling with relatively lower amounts of radiolabel (Fig. 3). It performs well across a range of enzyme concentrations, eliminating the need for careful enzyme titrations (Fig. 4). OptiKinase works well for labeling of double-stranded oligonucleotides (Fig. 5). It can also be used for phosphorylation of oligonucleotides with non-labeled ATP and for labeling of variety of double-stranded DNA ends after restriction digestion and removal of the 5'-phosphate by phosphatase treatment (data not shown).

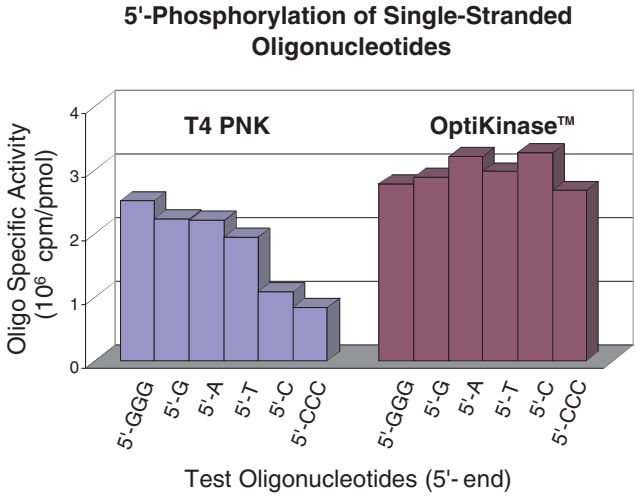

Fig. 2. Comparison of phosphorylation of oligonucleotides by OptiKinase versus T4 PNK. Oligonucleotides (5 pmol) and [γ-^{33}P]ATP (5 pmol) were treated with 10 units of T4 PNK or OptiKinase in 25 µl volume at 37°C for 30 min, followed by quantification of labeling. Oligonucleotides were identical except at their 5'-ends. Oligonucleotides corresponded to 31-mers (G, A, T, and C) or 18-mers (GGG and CCC).

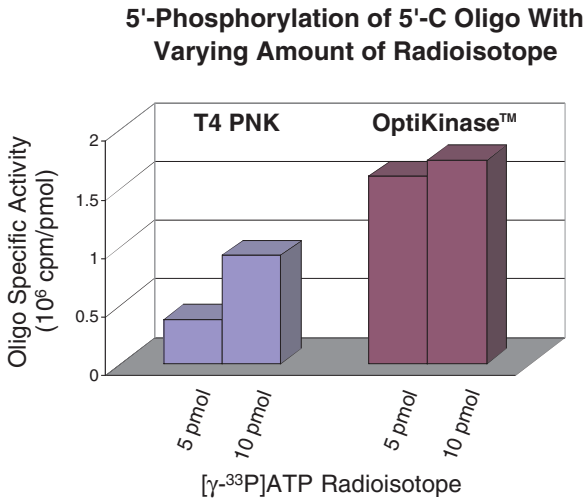

Fig. 3. Comparison of the effect of radiolabeled ATP concentration on the extent of phosphorylation by OptiKinase versus T4 PNK. 5'-C oligonucleotide (5 pmol) and [γ-^{33}P]ATP (5 pmol or 10 pmol) were treated with 10 units of T4 PNK or OptiKinase in 25 µl volume at 37°C for 30 min, followed by quantification of labeling.

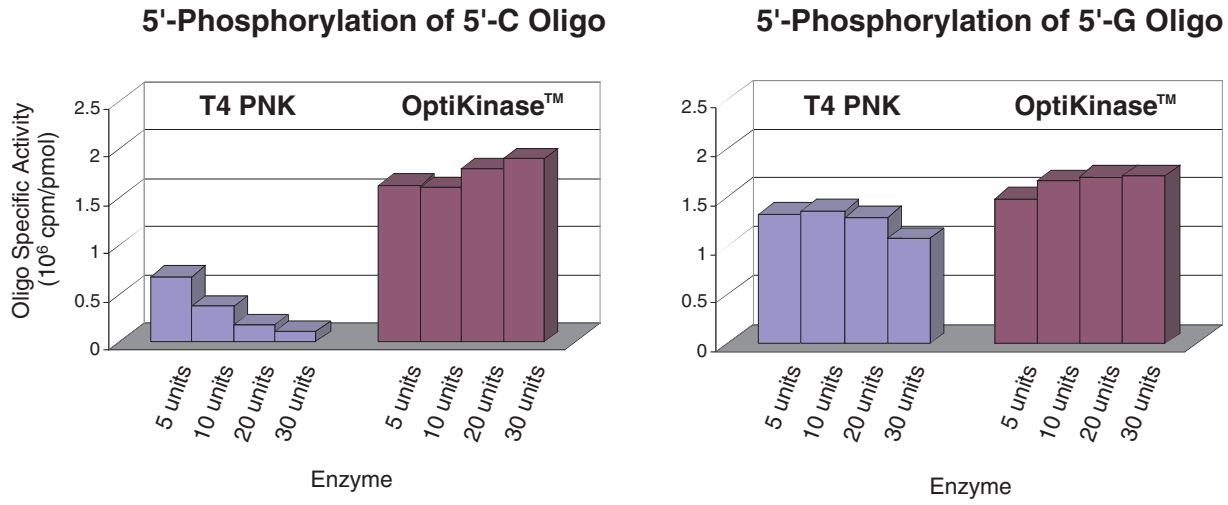

Fig. 4. Comparison of the effect of enzyme concentration on the extent of phosphorylation by OptiKinase versus T4 PNK. 5'-C or 5'-G oligonucleotides (5 pmol) and [γ-^{33}P]ATP (5 pmol) were treated with 5 to 30 units of T4 PNK or OptiKinase in 25 µl volume at 37°C for 30 min, followed by quantification of labeling.

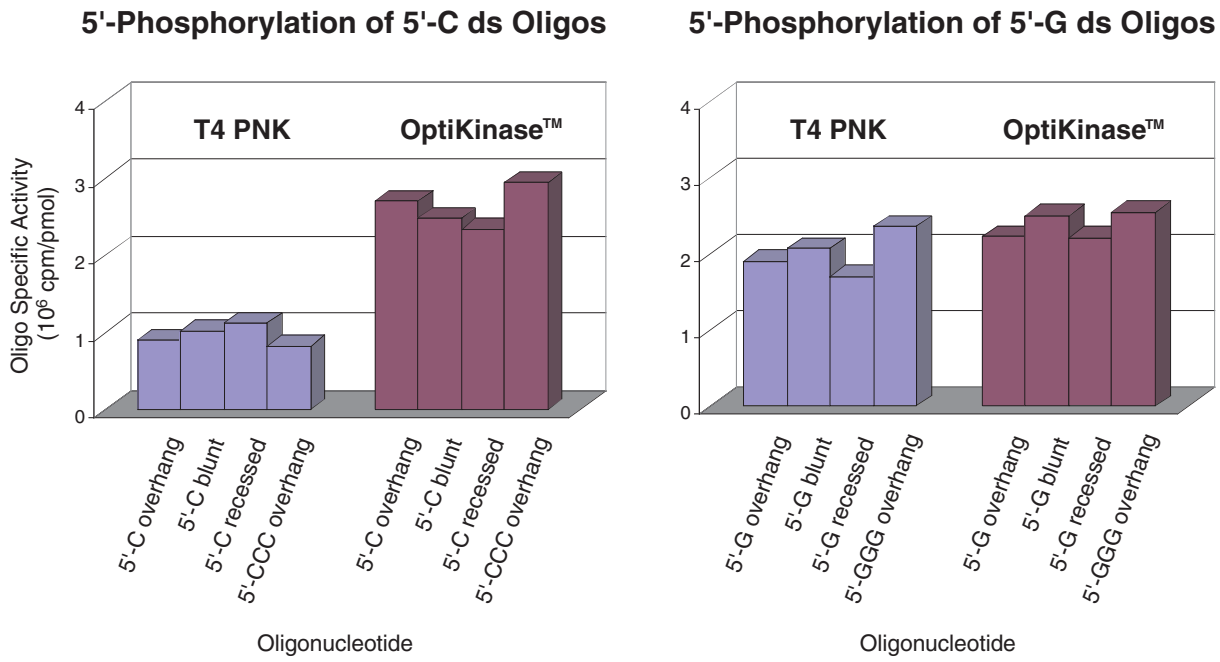

Fig. 5. Comparison of phosphorylation of double-stranded oligonucleotides by OptiKinase versus T4 PNK. 5'-C or 5'-G double stranded 18-mer oligonucleotides (5 pmol) and [γ-^{33}P]ATP (5 pmol) were treated with 10 units of T4 PNK or OptiKinase in 25 µl volume at 37°C for 30 min, followed by quantification of labeling.

Discussion

OptiKinase outperforms T4 PNK in a variety of 5'-labeling reactions, in terms of labeling uniformity, efficiency, and ease of use. For these reasons, OptiKinase is highly recommended for 5'-phosphorylation applications in general, and for 5'-labeling applications in particular.

References

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