

PRODUCT NUMBER: IAF170
50 reactions
LOT NUMBER: 425-0102-02



Ice and Fire[™] Phosphorylation Kit

Product components

The Kit includes the following components:

10X reaction buffer
SAP/PNK enzyme mixture
Phosphorylated ssDNA oligomer (10 pmol/ μ L) for use as positive control

Components not supplied with the kit:

Radiolabeled γ -ATP [³²P]
ATP
Nuclease free water

Introduction

The *Ice and Fire* Phosphorylation kit provides optimized reagents for the 5' end labeling of nucleic acids. The simple protocol utilizes low and high temperature to control the activity of thermolabile Shrimp Alkaline Phosphatase (SAP) and thermostable ThermoPhage[™] polynucleotide kinase (PNK) in one solution and optimized reaction buffer (patent pending). The kit is designed to label 5' ends of DNA or RNA molecules with terminal mono-, di- or triphosphates. Other nucleic acids such as PCR products, oligonucleotides, restriction-digested plasmid DNA, or complex sample RNA or DNA can also be labeled using the kit. The substrate can be of virtually any length.

Benefits

- Efficient 5' end labeling of phosphorylated nucleic acids, single- or double-stranded
- Simple temperature control of enzymatic activity
- No extra cleanup steps like phenol/chloroform or spin-columns are needed between the reactions, only heat denaturation of the SAP
- Single tube format, simplified procedure

Applications

5' - end label of phosphorylated DNA and RNA molecules for:

- Nuclease protection assays
- Hybridization blots
- Quantitative PCR

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Overview

Shrimp alkaline phosphatase digests mono-, di- and triphosphates from a 5' end of nucleic acids leaving a 5' hydroxyl group. PNK catalyzes the transfer of the gamma phosphate from ATP to the 5'-OH of a nucleic acid molecule. The combination of the two enzymes in a simplified temperature-controlled procedure allows for a rapid and efficient 5' end labeling of phosphorylated nucleic acids.

Nucleic acids with a 5'-phosphate needs to be dephosphorylated before the kinase reaction. The kit includes Shrimp Alkaline Phosphatase and thermostable polynucleotide kinase enzyme mixture. This allows for dephosphorylation of the nucleic acids at lower temperature followed by heat inactivation of the phosphatase prior to addition of radiolabeled [γ -³²P]ATP and labeling by the novel thermostable polynucleotide kinase (1). The SAP denaturation and the PNK reaction are done at 70°C. No SAP activity can be detected after the heating step, followed by the PNK reaction.

Nucleic acids with a 5'-hydroxyl (-OH) group can also be labeled using this kit. In this case the phosphatase is directly inactivated by heating followed by addition of gamma radiolabeled ATP and incubation at 70°C.

Protocol I

Protocol for 5' radio-labeling of nucleic acids

The *Ice and Fire* phosphorylation kit provides controls and optimized reagents for quick and easy end-labeling of DNA or RNA

1) Dephosphorylation of target nucleic acid (SAP reaction):

10x SAP/PNK buffer	2.5 μ L
SAP/PNK enzyme mix	1 μ L
Target DNA/RNA	10-100 pmol
Nuclease free water	up to 24 μ L

Incubate at 37°C for 60 minutes

2) Phosphorylation of target nucleic acid (PNK reaction):

After incubation at 37°C the sample is heated to 70°C. After 15 minutes at 70°C add X μ L radiolabeled [γ -³²P]ATP to the reaction equivalent to a 2-5 molar excess ATP over ends to be labeled (for example using 7,000 Ci/mmol, 150 mCi/ml [γ -³²P]ATP, 1 μ L is about 25 pmol). Incubate at 70°C for additional 30-60 minutes. Stop the reaction by cooling down to +4°C or put on ice.

Optional: Add 1 mM EDTA and/or heat the reaction at 95°C for 5 minutes to terminate the reaction.

Optional: Clean the probe by spin column (for example Qiagen nucleotide removal kit, Cat. No. 28304 or equivalent spin columns) or PAGE gel purification as described by Sambrook et al. (2).

The radiolabeled nucleic acids are now ready for use

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Protocol II

Protocol for 5' labeling of 5' hydroxylated nucleic acids

Prokaria Ltd provides ThermoPhage™ PNK also for labeling of 5' hydroxylated nucleic acids but the *Ice and Fire* phosphorylation kit can also be used for this kind of applications.

1) Reaction mixture

10x SAP/PNK buffer	2.5 µL
SAP/PNK enzyme mix	1 µL
Target DNA/RNA	10-100 pmol
Nuclease free water	upto 24 µL

Incubate at 70°C for 15 minutes to inactivate the SAP enzyme.

2) Polynucleotide kinase 5' labeling of target nucleic acid:

Add X µL radiolabeled [γ -³²P]ATP to the reaction equivalent to 2-5 molar excess ATP over ends to be labeled (for example using 7,000 Ci/mmol, 150 mCi/ml [γ -³²P]ATP, 1 µL is about 25 pmol). Incubate at 70°C for 60 minutes. Stop the reaction by cooling down to +4°C or put on ice.

Optional: Add 1 mM EDTA and/or heat the reaction at 95°C for 5 minutes to terminate the reaction.

Optional: Clean the probe by spin column (for example Qiagen nucleotide removal kit, Cat. No. 28304 or equivalent spin columns) or PAGE gel purifications as described by Sambrook et al. (2).

The radiolabeled nucleic acids are now ready for use

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Protocol III

Phosphorylation without radioactive ATP

This kit can also be used for phosphorylation of 5' hydroxylated nucleic acids prior to cloning procedure etc.

1) Reaction mixture

10x SAP/PNK buffer	2.5 μ L
SAP/PNK enzyme mix	1 μ L
Target DNA/RNA	1-500 pmol
Nuclease free water	upto 24 μ L

Incubate at 70°C for 15 minutes to inactivate the SAP enzyme.

2) Polynucleotide kinase 5' phosphorylation of target nucleic acid:

Add ATP to the reaction to a final concentration of 10-100 μ M (at least twice the concentration of nucleic acids ends to be phosphorylated. Incubate at 70°C for 60 minutes. Stop the reaction by cooling down to +4°C or put on ice.

Optional: Clean the probe by spin column (for example Qiagen nucleotide removal kit, Cat. No. 28304 or equivalent spin columns) or PAGE gel purifications as described by Sambrook et al. (2).

The nucleic acids are now ready for cloning procedure

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**Evaluation
Protocol**

If specific radioactivity needs to be determined or if the researcher wants to evaluate the efficiency of the reaction, the following evaluation protocol can be used. Note that scintillation counter, scintillation liquid and DE81 filters or equivalent instruments and components are required for this evaluation.

Dilute 1 μL of the reaction 1/10 and spot 1 μL to a Whatman Inc. DE81 filter (Cat no. 3658023) or its equivalent. As a positive control, same amount is put on one filter that does not undergo the washing steps. Reaction solution without enzyme mix can be used as negative control and washed with the samples. The sample and negative control filters are washed twice in 400 ml sodium phosphate buffer pH 7 for 30 minutes, dried and put into scintillation counter vials, scintillation liquid added and counted for radioactivity in an liquid scintillation counter.

To determine the specific activity of the labeled nucleic acid, calculate the total cpm incorporated in the reaction and divide it by the amount of nucleic acid labeled in pmol.

$$\frac{\text{\#total cpm incorporated} \times \text{dilution factor} \times \text{volume}}{\text{\#total pmol nucleic acid}}$$

Expected specific activity:

When $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ with a specific activity of 7000 Ci/mmol is used for the kinase reaction, the nucleic acid should be labeled to at least 1×10^6 cpm/pmol on the reference date. Decay of ^{32}P from the reference day stated on the vial of radiolabeled ATP, can be calculated as:

$$\frac{\text{original cpm}/\mu\text{l}}{2^{(\text{days}/14.3)}}$$

References

1. Blondal, T., Hjorleifsdottir, S., Aevarsson, A., Fridjonsson, O.H., Skirnisdottir, S., Wheat, J.O., Hermannsdottir, A.G., Hreggvidsson, G.O., Smith, A.V. and Kristjansson, J.K. (2005) *J Biol Chem*, **280**, 5188-5194.
2. Sambrook, J., Fritsch, E.F. and T., M. (1989) *Molecular cloning, a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor NY.