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# Polynucleotide Kinase

From *Escherichia coli* E. D. pFDX
*ATP: 5'-dephosphopolynucleotide 5'-phosphotransferase, EC 2.7.1.78*

**Cat. No. 10 174 645 001** 200 U (10 U/ µl)
**Cat. No. 10 633 542 001** 1,000 U (10 U/ µl)

**Version 19**
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Store at −15 to −25°C

## Product description

**Phosphorylation buffer 10x conc.** 10x Incubation buffer for direct phosphorylation, supplied with the enzyme:
500 mM Tris-HCl, 100 mM $MgCl_2$,1 mM EDTA, 50 mM dithioerythritol, 1 mM spermidine, pH 8.2 (at +25°C).

**Storage buffer** 50 mM Tris-HCl, 1 mM dithiothreitol, 0.1 mM EDTA, 0.1 µM ATP, 50% glycerol (v/v), pH approx. 7.5 (+25°C).

**Specific activity** > 40 x $10^3$ U/mg acc. to (1, 2).

**Unit definition** Polynucleotide Kinase (PNK) is assayed acc. to (1). One unit is the enzyme activity which catalyzes the incorporation of 1 nmol [$^{32}$ P] into acid-precipitable products within 30 min at +37°C.

**Properties** Polynucleotide Kinase catalyzes the transfer of the terminal phosphate group of ATP to the 5´-hydroxylated terminus of DNA or RNA (1). The enzyme also catalyzes the exchange of 5´-terminal phosphate groups (4).

**Application** Labeling of 5´-hydroxyl ends of DNA or RNA with [$\gamma^{32}$P]-ATP by direct phosphorylation or by an exchange reaction.

Alternatively, polynucleotide kinase can be used for removal of 3´-phosphates from RNA or DNA at low pH conditions without altering the structure of the 5´-terminus.

**Storage/ stability** The undiluted enzyme is stable at -15 to -25°C through the expiration date printed on the label.

## Standard assay

**Protocol** Procedure for 5´-end-labeling of free 5´-OH-termini.

| Step | Action |
|---|---|
| 1 | • Add to the dephosphorylated DNA-fragment solution (20 pmol 5´-OH-termini) on ice:<br><br>**Reagent** / **Vol.**<br>Phosphorylation buffer, 10x conc. / 2 µl<br>20 pmol [$\gamma$-$^{32}$P] ATP, 10 µmol (aqueous solution) / x µl<br>Add double-distilled water to a final volume of 19 µl. / x µl<br>10 U Polynucleotide Kinase / 1 µl<br><br>• Mix and centrifuge briefly.<br>• Incubate for 30 min at +37°C. |
| 2 | Stop the reaction by cooling in an ice bath. |

**Labeling yield** The yield of the labeling reaction can be determined by trichloracetic acid precipitation.

## Quality control

For lot-specific certificates of analysis, see section, **Contact and Support**.

## References


1 Richardson, C. C. (1968) Proc. Natl. Acad. Sci. USA **54**, 158.
2 Bradford, M. (1976) Anal. Biochem. **72**, 248.
3 Laemmli, U. K. (1970) Nature **227**, 680.
4 Chaconas, G. & v. d. Sande, J. H. (1980), Methods Enzymol. **65**, 680.


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**Changes to previous version** Update of the chapter Quality Control.

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