Phosphatase, alkaline

From calf intestine
Orthophosphoric-monoester phosphohydrolase (alkaline optimum)

Cat. No. 10 713 023 001 1,000 U (1 U/µl)
Cat. No. 11 097 075 001 1,000 U (20 U/µl)

Product Overview

<table>
<thead>
<tr>
<th>Content</th>
<th>1,000 U (1 U/µl), (Cat. No. 10 713 023 001) 1,000 U (20 U/µl), (Cat. No. 11 097 075 001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline Phosphatase</td>
<td>(alkaline optimum) Orthophosphoric-monoester phosphohydrolase From calf intestine</td>
</tr>
<tr>
<td>Dephosphorylation Buffer 10x</td>
<td>0.5 M Tris-HCl, 1 mM EDTA, pH 8.5 (+20°C)</td>
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</table>

Unit definition

Alkaline Phosphatase is assayed according to (1).

One unit of Alkaline Phosphatase is the enzyme activity which hydrolyzes 1 µmol of 4-nitrophenyl phosphate in 1 min at +37°C under assay conditions. 

**Note:** According to (1), 5 units Alkaline Phosphatase (+37°C; diethanolamine buffer) correspond to 1 unit Alkaline Phosphatase (+25°C; glycine/NaOH buffer).

Activity determination

The activity determination is performed according to (1) at +37°C in 1 M diethanolamine buffer, 10 mM 4-nitrophenyl phosphate, 0.5 mM MgCl₂, pH 9.8.

Specific activity

Approx. 2 U/µg according to (1) and (2).

Stability

Stable at +2 to +8°C until the expiration date printed on the label.

Procedures

**Dephosphorylation of DNA (3, 4)**

The reaction assay is adjusted with 1/10 volume 10× Dephosphorylation Buffer. 1 pmol 5' terminal phosphorylated DNA fragments (3'-recessed, 5'-recessed or blunt-ended) are incubated with 1 unit Alkaline Phosphatase at +37°C for 60 min.

**Dephosphorylation of RNA (3, 4)**

The reaction assay is adjusted with 1/10 volume 10× Dephosphorylation Buffer. 1 pmol 5' terminal phosphorylated RNA fragments are incubated with 1 unit Alkaline Phosphatase at +50°C for 60 min.

**Inactivation of Alkaline Phosphatase (4, 5)**

Add 1 / 10 volume of 200 mM EGTA, to the reaction assay and heat to +65°C for 10 min. To achieve complete inactivation of Alkaline Phosphatase, perform an extraction with phenol/chloroform/isoamylalcohol (50 : 48 : 2).

Quality Control

Lot-specific certificates of analysis are available at www.roche-applied-science.com/certificates.

**Absence of deoxyribonucleases**

1) 1 µg λDNA is incubated with Alkaline Phosphatase for 1 h at +37°C in 25 µl Dephosphorylation Buffer. For up to 50 U of Alkaline Phosphatase, no degradation of λDNA is detectable.

2) 1 µg Eco RI/Hind III fragments of λDNA is incubated with Alkaline Phosphatase for 1 h at +37°C in 25 µl Dephosphorylation Buffer. For up to 50 U of Alkaline Phosphatase, no alteration of the banding pattern is shown.

**Absence of nicking activities**

1 µg supercoiled pBR322 DNA is incubated with Alkaline Phosphatase for 1 h at +37°C in 25 µl Dephosphorylation Buffer. For up to 50 U of Alkaline Phosphatase, no relaxation of supercoiled structure of pBR322 is detectable.

**Absence of exonucleases**

15 nmol of sonicated [3H]-DNA (approx. 100,000 cpm/µg) from calf thymus are incubated with Alkaline Phosphatase for 4 h at +37°C in 100 µl buffer (50 mM Tris-HCl, 1 mM MgCl₂, 1 mM dithioerythritol, pH 7.5). For up to 50 U of Alkaline Phosphatase, no radioactivity is detectable.

**Absence of ribonucleases**

5 µg MS2 RNA is incubated with Alkaline Phosphatase for 1 h at +50°C in 50 µl Dephosphorylation Buffer. For up to 50 U of Alkaline Phosphatase, no degradation of MS2 RNA is detectable.

References


Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our Online Technical Support Site.

To call, write, fax, or email us, visit the Roche Applied Science homepage, www.roche-applied-science.com, and select your home country. Country-specific contact information will be displayed.