Dephosphorylation of 5' -ends of DNA using AnP (M0289)

Version 2

New England Biolabs

Abstract

This is the protocol for dephosphorylation of 5'-ends of DNA using AnP (Antarctic Phosphatase - M0289).

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Guidelines

Dephosphorylation of 5' -ends of DNA in Restriction Enzyme Reaction

- The phosphate can be added directly into the digestion reaction during or after DNA digestion
- Antarctic Phosphatase is active in all NEB restriction enzyme buffers only when supplemented with Antarctic Phosphatase Reaction Buffer, which provides Zn2+ required for enzyme activity
- The restriction enzyme should be heat inactivated at the same time as the phosphatase after digest and dephosphorylation
- If restriction enzyme cannot be heat inactivated, DNA purification is required before ligation

Materials

Antarctic Phosphatase - 1,000 units M0289S by New England Biolabs

Protocol

Step 1.

Prepare a 20 µl reaction as follows:

DNA	1 pmol of DNA ends*
Antarctic Phosphatase Reaction Buffer (10X)	2 µl
Antarctic Phosphatase	5 units
H_2O, purified	to 20 µl**

NOTES

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** Note: 1 pmol of DNA ends is about 1 µg of a 3 kb plasmid.*
*** Scale larger reaction volumes proportionally.*

Step 2.

Incubate at 37°C for 30 minutes.

DURATION
00:05:00

Step 3.

Stop reaction by heat-inactivation at 80°C for 2 minutes.