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Dephosphorylation of 5´-ends of DNA using Antarctic Phosphatase (NEB #M0289) V.3

New England Biolabs[1]

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New England Biolabs (NEB)
Tech. support phone: **+1(800)632-7799** email: **info@neb.com**

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

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https://www.neb.com/protocols/0001/01/01/vector-dephosphorylation-protocol

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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 **New England Biolabs Catalog #M0289S**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

1 

Prepare a **20 µL** reaction as follows:

A	B
COMPONENT	**AMOUNT**
DNA	1 pmol of DNA ends*
Antarctic Phosphatase Reaction Buffer (10X)	2 µl
Antarctic Phosphatase	5 units
H2O, purified	to 20 µl**

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

2 

Incubate at **37 °C** for **00:30:00** .

3 Stop reaction by heat-inactivation at **80 °C** for **00:02:00** .

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