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Dephosphorylation of 5'-ends of DNA using rSAP (M0371) V.2

New England Biolabs¹¹New England Biolabs

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dx.doi.org/10.17504/protocols.io.bg9kz4w**New England Biolabs (NEB)**Tech. support phone: **+1(800)632-7799** email: **info@neb.com****New England Biolabs**
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Protocol for Dephosphorylation of 5'-ends of DNA using rSAP (M0371).

DOI

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dephosphorylating 5' ends of DNA, Dephosphorylation using rSAP, phosphatase, dephosphorylate  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
- rSAP is active in all NEB restriction enzyme buffers
- The restriction enzyme should be heat inactivated at the same time as the phosphatase after digest and dephosphorylation
- If restriction enzyme(s) cannot be heat inactivated, DNA purification is required before ligation

MATERIALS

 [Shrimp Alkaline Phosphatase \(rSAP\) - 500 units](#) **New England**

Biolabs Catalog #M0371S

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

1



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

A	B
DNA	1 pmol of DNA ends*
CutSmart® Buffer (10X)	2 µl
rSAP	1 unit
H2O, purified	to 20 µl**

* 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  **65 °C** for  **00:05:00** .