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Dephosphorylation of 5´-ends of DNA using rSAP in Restriction Enzyme Reaction (M0371) V.4

New England Biolabs[1]

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This protocol explains methods for dephosphorylation of 5´-ends of DNA using rSAP in restriction enzyme reaction (M0371).

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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

Digest **1 µg** – **5 µg plasmid DNA** in a 20 µl reaction as follows:

A	B
DNA	≥ 1 µl
Restriction Enzyme Buffer (10X)	2 µl
Restriction Endonuclease	1 µl
H_2O, purified	to 20 µl**

Scale larger reaction volumes proportionally.

2

Incubate at **37 °C** for **01:00:00** or follow manufacturer's recommendations.

3

Add **1 unit** of rSAP **for every** **1 pmol** of DNA ends (about **1 µg** of a 3 kb plasmid).

4

Incubate at **37 °C** for 30-60 minutes.

5 Stop reaction by heat-inactivation of rSAP and restriction enzyme (follow manufacturer's recommendations).

If restriction enzyme cannot be heat-inactivated, DNA purification is required before ligation.

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