Dephosphorylation using rSAP in Restriction Enzyme Reaction(M0371) Version 3

New England Biolabs

Abstract

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

Citation: New England Biolabs Dephosphorylation using rSAP in Restriction Enzyme Reaction(M0371). **protocols.io** dx.doi.org/10.17504/protocols.io.nkxdcxn
Published: 02 Mar 2018

Materials

Shrimp Alkaline Phosphatase (rSAP) - 500 units M0371S by New England Biolabs

Protocol

Step 1.

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

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

PROTOCOL

. Reaction Mixture for M0371

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NOTES

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Scale larger reaction volumes proportionally.
Step 1.1.
DNA ≥ **1 µl**

Step 1.2.
Restriction Enzyme Buffer (10X) **2 µl**

AMOUNT

2 µl Additional info:

Step 1.3.

Restriction Endonuclease **1 µl**

AMOUNT

1 µl Additional info:

Step 1.4.

H2O, purified to **20 µl**

Step 2.

Incubate at 37°C for 60 minutes or follow manufacturer’s recommendations.

DURATION

01:00:00

Step 3.

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

Step 4.

Incubate at 37°C for 30–60 minutes.

DURATION

01:00:00

Step 5.

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

NOTES

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

Warnings

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