

FAQs for DNA End Modification: Dephosphorylation

1. Does the DNA need to be purified after a restriction digest and prior to the dephosphorylation step?

If the enzymes used in the restriction digest are heat inactivatable, then a purification step is not needed. If the enzymes used are not heat-inactivatable, then a clean-up step between the restriction digest and the dephosphorylation step is recommended.

2. Does the DNA need to be purified after the dephosphorylation step and prior to the ligation step?

If you use rSAP or Antarctic Phosphatase, which are heat-inactivatable, then a clean-up step is not necessary. If the alkaline phosphatase used is CIP, which is not heat-inactivatable, then a clean-up step is necessary.

3. Which alkaline phosphatase, CIP, Antarctic or rSAP, works best?

rSAP ([NEB #M0371](#)) is a superior choice because it combines the advantages of both CIP ([NEB #M0290](#)) and Antarctic Phosphatase ([NEB #M0289](#)). rSAP has a very high specific activity comparable to CIP, but CIP cannot be completely heat-inactivated. rSAP is heat-inactivated in 5 minutes, as is Antarctic Phosphatase, but rSAP is preferred over Antarctic Phosphatase because it does not require added zinc or any other co-factors. As a result, rSAP can be added directly to restriction enzyme digests. Additionally, after heat-inactivation of rSAP, it is not necessary to purify vector DNA prior to the ligation reaction.

4. Are the alkaline phosphatases active in NEBuffers?

rSAP: rSAP is active in all restriction enzyme [NEBuffers 1.1](#), [2.1](#), [3.1](#) and [CutSmart™ Buffer](#), and can be added directly to digested DNA.

Antarctic Phosphatase: Antarctic phosphatase has a strict requirement for Zinc and has optimal activity at pH 6.0. Antarctic Phosphatase can be used in NEBuffers 1, 2, 3 or 4 as well as the unique NEBuffers for EcoRI and BamHI only when supplemented with 10X Antarctic Phosphatase Reaction Buffer to a final concentration of 1X.

CIP: CIP will work in NEBuffers 2, 3, or 4, as well as the unique NEBuffer EcoRI. NEBuffer 3 gives optimum activity. Add 2X more CIP if the buffer contains less than 50 mM salt.

5. Why do some protocols recommend using CIP at 50°C and some recommend 37°C?

It has been shown that DNA fragments with 3' extensions or blunt ends are slightly more difficult to dephosphorylate with CIP than those fragments with 5' extensions. CIP works slightly better on 3' extensions and blunt-ends at 50°C. We have found that the difference in efficiency is about 20-25%. Some protocols also recommend the

addition of more CIP for these difficult ends. For the sake of simplicity, we recommend 37°C and an enzyme concentration of 0.5 unit/μg in a 10 μl reaction for all types of ends.