

FAQs for DNA End Modification: Blunting

1. I've blunted my DNA with the Quick Blunting Kit and now need to dephosphorylate the reaction with an alkaline phosphatase. Do I need to purify the reaction?

Yes, the reaction will need to be purified prior to dephosphorylation with Antarctic Phosphatase, rSAP, CIP or any other commercially available phosphatase. The reaction can be purified using a commercial purification kit, phenol extraction/ethanol precipitation or gel electrophoresis.

2. Does the PCR product need to be purified before blunting with the Quick Blunting Kit? What methods can be used to purify the product?

Yes, PCR reactions should be purified before blunting. Any PCR column purification will work.

3. I've digested my DNA and now want to blunt directly with the Quick Blunting Kit without purifying, can I add the blunting reagents directly to the restriction digest?

We recommend to heat inactivate the restriction enzyme after a restriction digest and prior to the blunting reaction. If the restriction digest has been carried out with enzymes that can be inactivated by a heat treatment, then after the heat inactivation step, add 0.1 volume of dNTPs and 1 ul of Blunting Enzyme and incubate at room temperature for 15 minutes. If the restriction enzymes used are not inactivated by a heat treatment, then the reaction will need to be purified using a commercial purification kit, phenol extraction/ethanol precipitation or gel electrophoresis prior to the blunting step.

4. I've accidentally skipped the heat-kill step after the blunting reaction with the Quick Blunting Kit. Will the ligation still work?

Yes, but there may be a higher background since the polynucleotide kinase will still be active and can phosphorylate the vector.

5. I've blunted my sonicated gDNA with the Quick Blunting Kit for 15 minutes instead of the recommended 30 minute incubation time, will the reaction still work?

A slightly lower transformation efficiency is seen with shorter incubations but the reaction will still work. In general, 1.5 X fewer transformants are seen when PCR product or sheared/nebulized DNA is incubated for 15 minutes instead of 30 minutes. If a 15 minute incubation time is desired, increase blunting mix to 2 μ l in the reaction.

6. What enzymes can be used for removing 3' overhangs and filling in 5' overhangs (3' recessed ends)?

DNA Polymerase I, Large (Klenow) Fragment ([NEB# M0210](#)) and T4 DNA Polymerase are the best choices for this application. DNA Polymerase I, Large (Klenow) Fragment

can be used at 25°C or room temperature, but T4 DNA Polymerase must be used at 12°C due to its robust exonuclease. Both work well in a wide variety of buffers.

7. Can T4 DNA Polymerase be used in other NEBuffers?

When supplemented with dNTPs (not included), T4 DNA Polymerase is fully active in NEBuffers 2.1 and CutSmart, as well as NEBuffers 2 and 4 when supplemented with BSA. It is also active in T4 DNA Ligase Reaction Buffer when supplemented with BSA. There is an approximate 25% loss of activity in NEBuffers 1.1 and 3.1 as well as NEBuffers 1 and 3 when supplemented with BSA.

8. What are the main causes of blunting reaction failure using T4 DNA Polymerase?

The following conditions can cause the exonuclease to overwhelm the polymerase activity causing recessed instead of blunt ends:

- *Adding too much enzyme
- *Incubating for too long
- *Incubating at greater than 12°C
- *Failure to add nucleotides or the nucleotide level is too low
- *Heat inactivating without EDTA

9. Is T4 DNA Polymerase active at room temperature?

We suggest 12°C. The DNA ends "breathe" at higher temperatures allowing the exonuclease to remove nucleotides past blunt.

10. Are the nucleotides needed to remove a 3' overhang using T4 DNA Polymerase or DNA Polymerase I, Large (Klenow) Fragment?

Yes, T4 DNA Polymerase will remove bases past blunt if the nucleotide concentration is less than 100 µM. In the absence of dNTPs DNA Polymerase I, Large (Klenow) Fragment will remove more nucleotides than necessary to blunt.

11. What are the main causes for blunting reaction failure using DNA Polymerase I, Large (Klenow) Fragment?

The following conditions can cause the exonuclease to overwhelm the polymerase activity causing recessed instead of blunt ends:

- *Adding too much enzyme
- *Incubating for too long
- *Incubating at temperatures greater than 25°C
- *Failure to add nucleotides or the nucleotide level is too low
- *Heat inactivating without EDTA.

12. Can DNA Polymerase I, Large (Klenow) Fragment be used in other NEBuffers?

Yes, Klenow is active in all NEBuffers (1, 1.1, 2, 2.1, 3, 3.1, 4, CutSmart) and T4 DNA Ligase Buffer, but optimal activity is observed in NEBuffer 2. All NEBuffers must be supplemented with dNTPs.

13. What enzyme can I use to remove 5' overhangs?

Mung Bean nuclease can remove 5' overhangs. However this enzyme is a very active nuclease, so some optimization may be required to use it in this application.

14. Will Mung Bean Nuclease degrade double stranded DNA?

When used at temperatures higher than the 30°C recommended, or for longer periods, degradation of the substrate DNA will result. Further, since the endonucleic activity is known to occur at 'bubbles' on the DNA helix, which transiently form along the length of the molecule, substrates which are already nicked, or are of high AT content are more susceptible to this endonucleic activity.

15. What adjustments in protocol are recommended when using Mung Bean Nuclease for blunting?

The recommended temperature of incubation is room temperature, since higher temperatures may cause sufficient breathing of the dsDNA ends that the enzyme may degrade some of the dsDNA sequence. The number of units to be used and time incubation may be determined empirically to obtain best results.

16. Is Mung Bean Nuclease active in other NEBuffers?

Mung Bean Nuclease is active in NEBuffers 1, 2, and 4. Longer incubations may be required when using these buffers.

17. Can Mung Bean Nuclease be heat inactivated?

DO NOT ATTEMPT TO HEAT INACTIVATE! Although Mung Bean Nuclease can be inactivated by heat, this is not recommended because the DNA begins to "breathe" before the Mung Bean Nuclease is inactivated and undesirable degradation occurs at breathing sections. Purify DNA by phenol/chloroform extraction and ethanol precipitation or spin column purification.