

5'-Phosphorylation of Oligonucleotides prior to using Phusion Site-Directed Mutagenesis Kit (F-541)

Overview

Protocol

1. Add the following components to a microcentrifuge tube:
 - 300 pmol oligonucleotide
 - 5 μ l 10X T4 Polynucleotide Kinase Reaction Buffer
 - 1 μ l T4 Polynucleotide Kinase 10 U/ μ l (NEB# M0201S/L)
 - 5 μ l 10 mM ATP
 - H₂O to a final volume of 50 μ l
2. Incubate the reaction at 37°C for 30 minutes.
3. Inactivate the T4 Polynucleotide Kinase at 65°C for 20 minutes. Alternatively PNK reaction can be phenol-extracted and ethanol precipitated or purified with a commercial kit.
4. The reaction products can be stored at -20°C or added directly to the mutagenesis reaction.