End-labeling Protocol

Overview

Introduction

5´ overhangs can be end-labeled radioactively with T4 Polynucleotide Kinase (PNK) , or filled in using the Klenow fragment with labeled dNTPs:
-1 µl T4 PNK
-1 µl ^{32}P ATP (3,000 Ci/mmol, 5 mCi/ml)
-2 µl T4 PNK Reaction Buffer (10X)
-1 or 2 µl DNA ladder (1 µg)

Protocol

1. Add distilled water to a reaction volume of 20 µl.
2. Incubate for 30 minutes at 37°C.
3. Run the samples for 50 to 60 minutes at 100V in TBE buffer in a 4-20% acrylamide gel (10 cm x 10 cm). A 20 minutes exposure gives very readable signals. The signal strength is about twice that signal when ADP is added to 100 µM.