

3' End Labeling of RNA using T4 RNA Ligase 1

Materials Required but not Supplied

T4 RNA Ligase 1 (ssRNA Ligase)

- Nuclease-free Water (NEB #B1500)
- RNase Inhibitor, Murine (NEB #M0314) (optional)

Overview

Introduction

Set up the labeling reaction at room temperature until the ligase is added to avoid DMSO precipitation. The addition of DMSO is essential.

Protocol

1. Set up a 30 μ l reaction as follows:
 - 1 X Reaction Buffer (50 mM Tris-HCl, pH 7.8, 10 mM MgCl₂, 1 mM DTT)
 - 1mM ATP
 - 1 μ g RNA
 - 10 % (v/v) DMSO
 - 1 μ M [³²P]pCp
 - 1 μ l (10 units) T4 RNA Ligase
2. Incubate overnight at 16°C