Typical Protocol for RNA Ligation (NEB #M0458)

Overview

1. Prepare 1 mM GTP by diluting the 10 mM stock in nuclease-free water. Store at –20°C for repeated use.

2. Assemble the following reaction in a nuclease-free PCR tube on ice:

COMPONENTS	AMOUNT
3´-phosphate RNA donor	10 pmol (0.5 pmol/µl)
5´-OH RNA acceptor	10 pmol (0.5 pmol/µl)
RtcB Reaction Buffer (10X)	2 µl
1 mM GTP	2 µl
10 mM $MnCl_2$	2 µl
RtcB RNA Ligase	1 µl (15 pmol)
Nuclease-free Water	Up to 20 µl

3. Incubate at 37°C for 1 hour.

4. We recommend cleaning up your reactions before moving on to downstream applications. This can be achieved by using a spin column-based method or phenol:chloroform extraction followed by ethanol precipitation.