NEW ENGLAND Biolabs

Faustovirus Capping Enzyme FCE Standard Capping Protocol (NEB #M2081)

Overview

Cap-0 Synthesis using FCE

Introduction

This protocol (50 µl reaction volume) is suitable for capping 50 to 100 ug of RNA using 50 units of FCE. The reaction can be scaled proportionately as needed to accommodate more or less RNA input.

NOTE: Capping efficiency is strongly influenced by 5′ end accessibility of the RNA substrate. For example, 50 units of FCE fully caps 200 µg of some substrates with unstructured 5' ends in a 100 µl reaction. If capping yield for 50 ug of RNA is satisfactory using 50 units of FCE, the reaction scale can be further optimized to cap more RNA while using the same 50 units of FCE. We recommend scaling the reaction volume proportionately for larger amounts of RNA (*e.g.*, 100 µl for 200 µg of RNA).

For highly structured substrates, more capping enzyme and longer reaction times may be required to yield complete capping. Additionally, increasing the reaction temperature can also improve capping yields.

A method for measuring capping efficiency is detailed in the following article: https://rnajournal.cshlp.org/content/28/8/1144

Protocol

1. Combine RNA and Nuclease-free H2O to a final volume of 38.0 µl.

2. (Optional) Heat at 65°C for 5 minutes.

3. Place tube on ice.

4. Add the following components in the order specified:

Reaction Mix	[Final]	Volume
RNA (from above)		38 µl
10X Capping Buffer	1X	5 µl
SAM (2 mM, diluted from 32 mM stock)	0.1 mM	2.5 µl
GTP (10 mM)	0.5 mM	2.5 µl
FCE (25 U/ul)		2 µl
Total		**50 µl**

5. Incubate at 37°C for 30 minutes.
6. RNA is now capped and ready for use in downstream applications. Some applications may require RNA to be purified prior to use. If the RNA needs a poly(A) tail, NEB Poly(A) Polymerase (NEB #M0276) can be used.

NOTE: The optional 65°C heating step is intended to reduced RNA secondary structure prior to capping. This step is not necessary for all substrates and can be omitted.