PRODUCT INFORMATION

mRNA Cap 2'-O-Methyltransferase

v. 230601

Catalog number

C15038-2000U

Package & Component

Name	Amount
mRNA Cap 2´-O-Methyltransferase (50,000 U/mL)	2000 U (1 vial)
10X Capping Enzyme Reaction Buffer	100 µL (1 vial)

Description

mRNA Cap 2´ O Methyltransferase specifi cally requires a Cap 0 structure (m7Gppp5'N) as a substrate, which synthesis from in vitro transcripts mRNA and capping enzyme modify.
This enzyme utilizes a methyl donor such as SAM to add a methyl group at the 2'-O position forming cap-1 structure.

Source

Escherichia coli

Purity

>95% as determined by SDS-PAGE. Purified by Ni-NTA chromatography.

Unit Definition

One unit is defined as the amount of enzyme required to methylate 10 pmoles of 80 nt long capped RNA transcript in 1 hour at 37°C.

Reaction Condition

1X Capping enzyme reaction buffer, supplemented with 0.5 mM GTP and 0.1 mM S-adenosylmethione (SAM). Incubate at 37°C.
10X Capping enzyme Reaction Buffer: 500 mM Tris-HCl (pH 8.0), 50 mM KCl, 10 mM $MgCl_2$, 10 mM DTT.

Storage Buffer

Capping enzyme is supplied in 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton® X-100 and 50% Glycerol.

Storage

Stored at -20°C. Avoid repeated freeze/thaw cycles.

Manuel

One-Step Capping and 2'-O-Methylation procedures:

1. Combine RNA and nuclease-free H_2O to a final 14 µL.
2. Heating at 65°C for 5 minutes then chill on ice for 5 minutes.
3. Below reaction mixture should be prepared **on ice** and combined in the following order:

Component	Amount	Final concentration
Denatured uncapped RNA	14 µL	-
10X Capping Buffer	2 µL	1X
10 mM GTP	1 µL	0.5 mM
4 mM SAM	1 µL	0.2 mM
Vaccinia Capping Enzyme	1 µL	10 U/rxn
mRNA Cap 2´-O-Methyltransferase	1 µL	50 U /rxn

	4. Gently mix the reaction thoroughly to achieve uniform distribution. 5. Incubate at 37°C for 60 minutes (For RNA less than 200 nt long increase incubation time to 2 hours).
Notes	Reaction should be performed under RNase free condition. Use nuclease-free tubes, reagents and water to avoid RNase contamination. Also, wear gloves when working with RNA.

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