



RNA production kit

Capping kit

Ready-to-use kit to add a Cap0 or Cap1 structure on up to 2.5 mg mRNA.

Protocol

Package content	Cap-K25 : Number of reactions : 25
Shipping conditions	The kit is shipped in dry-ice.
Storage conditions	Upon receipt and for long-term use, store the kit at -20°C.
Stability	1 year at -20°C.

For additional information and protocols
(optimization, scaling, co-transfection...)
tips, troubleshooting or other applications



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Any questions?



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Notes :

1. Technology

1.1 Description

This kit is designed for **the *in vitro* enzymatic capping of mRNA**. It allows the addition of a Cap0 or Cap1 structure on the 5' terminus of mRNA, improving its stability and translation.

The Vaccinia virus Capping Enzyme catalyzes the addition of a 7'methylguanylate cap to the 5' end of mRNA (Cap0) while the 2'O-methyltransferase uses the Cap0-mRNA as substrate to produce a Cap1 mRNA by methylation of 2'OH of the first nucleotide using S-Adenosylmethionine (SAM) as methyl group donor.

1.2 Kit composition

Components	Quantity	Storage
Ribonuclease inhibitor (40u/μl)	50 μl	-20°C
2'O-Methyltransferase (50u/μl)	50 μl	-20°C
Vaccinia Capping Enzyme (10u/μl)	50 μl	-20°C
Capping Buffer 10X	500 μl	-20°C
GTP (10mM)	500 μl	-20°C
SAM (32mM)	50 μl	-20°C
Nuclease-free water	2 ml	-20°C

Material needed not provided with the kit :

- mRNA to be capped.
- 70% ethanol for RNA washing.
- Nuclease-free buffers (1mM citrate buffer pH 6.4, TE buffer or RNase-free water) for RNA resuspension.
- Materials or kits for purification of the RNA product.

2. Applications and protocols

2.1 General Considerations

- SAM is unstable at pH 7-8 and 37°C: It should be thawed at 4°C and kept on ice during the experiment.
- If a Cap0 structure is needed, only use the Vaccinia Capping Enzyme and replace the amount of 2'O-methyltransferase by the equal amount of Nuclease-free water.
- RNAs are very sensitive to degradation and these simple precautions can limit their degradation:
 - Wear clean gloves and lab coat.
 - Clean work area and pipettes with a surface decontamination agent or with 0,5% SDS and Rinse thoroughly with RNase-free water.
 - Only use dedicated RNase-free tubes, barrier filter pipette tips and RNase-free water.

Important information :

Keep as much as possible your RNA sample on ice.

Unit definition :

- Vaccinia Capping Enzyme : one unit is defined as the amount of enzyme required to incorporate 10 pmol of GTP into an 80 nucleotides transcript in 1 hour at 37°C under standard assay conditions.
- 2'O-methyltransferase : one unit is defined as the amount of enzyme required to methylate 10 pmol of a 80 nucleotides Cap0 transcript in 1 hour at 37°C under standard assay conditions.
- RNase inhibitor: one unit is defined as the amount of enzyme required to reduce by 50% the activity of 5 ng of RNase A.

2.2 General protocol

This kit is designed to add a Cap1 (or Cap0) to 100 µg of mRNA in a 200µl reaction. To scale-up reaction, adjust the volumes accordingly.

Step 1 : RNA Denaturation (152 µl)

Dilute 100µg of RNA in 152µL of RNase-free water.

Heat the RNA at 65°C for 5 minutes.

Immediately after, place the denatured RNA on ice.

Step 2 : Reaction mix (48 µL)

Add sequentially the components described in the table below:

Components	Amount
Capping Buffer 10X	20 µl
GTP (10mM)	20 µl
SAM (32mM)	2 µl
Ribonucleases inhibitor	2 µl
2'O-methyltransferase	2 µl
Vaccinia Capping Enzyme	2 µl

Mix the heat-denatured RNA (Step 1) with reaction mix (Step 2). **Total Volume: 200µL.**

Incubate at 37°C for 2 hours.

NOTE: This time can be shortened to 30 min if your RNAs seems prone to degradation.

Step 3 : RNA Purification

Capped RNA can now be purified using your method of choice (organic extraction methods or commercially available RNA purification Kits). We suggest the precipitation method as follow:

- Precipitate the RNA by adding 1.5 volume of Nuclease-free water and 1.5 volume of precipitation solution (Lithium Chloride 7.5M, EDTA 0.05M, pH8), for a 200µL reaction mix: add 300 µL of the respective solutions.
- Incubate over night at -20°C.
- Centrifuge the tubes at 16000xg at 4°C for 15 minutes.
- Carefully discard the supernatant without impairing the pellet.

- Wash the pellet with 500 µl of 70% ethanol.
- Centrifuge again at 16000xg at 4°C for 5 minutes.
- Remove the ethanol and allow the pellet to dry.
- Resuspend the pellet in Nuclease-free water or buffer of your choice.

2.3 Troubleshooting

Ineffective capping :

- Check the purity of your mRNA. Use a purification method that remove protein and other contaminants (organic extraction method, spin column purification, lithium chloride precipitation).
- SAM can degrade over time at room temperature. Thaw SAM on ice and keep it at 4°C (melting ice or cooling rack) during the whole capping time.
- RNA may have secondary structure at the 5' end. You can try to modify the denaturation step condition by varying temperature over time (65°C for 20 minutes, 75°C for 10 minutes, 85°C for 5 minutes).
- Increase the capping reaction time (up to 3h) especially for small RNA < 200 nucleotides.

White precipitate in capping buffer :

- Heat the capping buffer at 37°C, homogenize and store at -20°C.

Additional products for your mRNA production and transfection experiments :

- **IVT Kit** for RNA production by *In Vitro* transcription
- **Poly(A) tailing Kit** for RNA polyadenylation
- **RmesFect** for mRNA transfection

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