Biochemical Assays for MTase Activity

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[Abstract] Methyltransferase (MTase) transfers a methyl group (-CH_3) from the donor S-adenosyl-L-methionine (AdoMet or SAM) to biologically active molecules such as hormones, neurotransmitters, lipids, proteins and nucleic acids. The addition of a methyl group causes a change in the physicochemical properties of the molecules. The mRNA cap structure is essential for cell and virus. Guanine-N7-methyltransferase (N7-MTase) methylates the GpppN cap at the N7 position of guanine, resulting in cap-0 structure (m7GpppN), and Ribose 2'-O-MTase further methylates the first nucleotide of higher eukaryotic cellular and viral mRNAs at the ribose 2'-OH position to form cap-1 (m7GpppNm) structures. Here, we describe a biochemical assay to detect the activities of mRNA capping MTases.

Materials and Reagents

1. Bodicon m7G capping system (Bodicon, catalog number: CS0130)
2. S-adenosyl methionine (SAM) (involved in Bodicon m7G capping system) (Bodicon, catalog number: CS0130)
3. Bodicon Capping Enzyme (10 U/μl) (involved in Bodicon m7G capping system) (Bodicon, catalog number: CS0130)
   Note: Because the sale of this kit was low, the previous companies which provide this capping kit were out of service. This capping kit was provided by a new company in China as custom-made products (contact e-mail: service@bodicon.cn, phone: +86-13628662011). In fact the similar capping kit from any other companies (such as Epicentre Biotechnologies, ScriptCap m7G capping system, catalog number: SCCE0610) is suitable for this experiment, and people can also contact with us to get the related protein or kit.
4. Inorganic pyrophospatase (YIPP) (New England Biolabs, catalog number: M2403S)
5. S-adenosyl [methyl-^{3}H] methionine ([^{3}H]-SAM) (PerkinElmer, catalog number: NET155H001MC)
6. DEAE Sephadex (GE Healthcare, catalog number: 17-0170-01)
7. GTP (Thomas Scientific, catalog number: R0461)
8. RNase inhibitor (Thomas Scientific, catalog number: EO0381)
9. RNase free water
10. Phenol-chloroform (pH 4.8-5.2 for RNA only)
11. Ethanol (RNase free)

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12. RNase free water
13. Sodium Dodecyl Sulfonate (SDS)
14. Ethylene Diamine Tetraacetic Acid (EDTA)
15. NH_4HCO_3
16. NaCl
17. 10x MTase Buffer (see Recipes)
18. Cap-0 cap structure (m7GpppN-RNA) (see Recipes)
19. Non-methylated Cap-0 cap structure (GpppN-RNA) (see Recipes)
20. MTase assay reaction mix (see Recipes)

Equipment

1. Bechtop
2. Water bath
3. Centrifuge
4. Liquid scintillation counter

Procedure

1. Synthesis of RNA cap structure as substrates.
   The nascent RNA transcribed *in vitro* possesses a 5' triphosphate end. Two RNA capping systems are used to synthesize cap structure as the substrates of MTase.
   a. Combine 1-10 µg RNA and RNase free water up to 12.5 µl of total reaction volume.
   b. To heat to denature the *in vitro* transcribed RNA, incubate the tube at 65 °C for 10 min, and transfer the tube to ice immediately.
   c. Combine the following reaction components in the order given:
      Cap-0 cap structure (m7GpppN-RNA)

Heat denatured RNA	12.5 µl
10x Bodicon Capping Buffer	2 µl
10 mM GTP	2 µl
3 mM SAM	1 µl
RNase inhibitor 40 U/µl	0.5 µl
Bodicon Capping Enzyme (10 U/µl)	2 µl
Total	20 µl

Non-methylated Cap-0 cap structure (GpppN-RNA)

Heat denatured RNA	12.5 µl
10x Bodicon Capping Buffer	2 µl
10 mM GTP	2 µl

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Inorganic pyrophospatase 0.1 U/μl	1 μl
RNase inhibitor 40 U/μl	0.5 μl
Bodicon Capping Enzyme (10 U/μl)	2 μl
Total	20 μl

d. Incubate at 37 °C for 2 h.
e. Purify the RNA substrates by using phenol-chloroform extraction and ethanol precipitation methods.

2. Prepare 10x MTase Buffer. The MTase buffer may be changed depending on different interested MTases.
3. Combine the following reaction components in the order given for MTase assays:

Purified MTases	1 μg
10x MTase Buffer	3 μl
GpppN-RNA or m7GpppN-RNA	2 μg
3 mM SAM	0.5 μl
[^{3}H]-SAM (67.3 Ci/mmol, 0.5 μCi/μl)	1 μl
RNase inhibitor 40 U/μl	1 μl
RNase free water	up to 30 μl
Total	30 μl

4. Incubate the reaction at 30-37 °C depending on different MTases for 1.5 h.
5. Transfer the tubes onto ice and add equal volume (30 μl) of 0.2% SDS, 20 mM EDTA.
6. Keep the tubes on ice, add 1 ml of 10 mM NH_4HCO_3 (pH 8.5).
7. Prepare 1 ml DEAE Sephadex column and equilibrated with 10 ml 10 mM NH_4HCO_3 (pH 8.5).
8. Load the samples onto the equilibrated column.
9. Wash the column with 10 ml of 10 mM NH_4HCO_3 (pH 8.5), 100 mM NaCl.
10. Elute the samples with 1.5 ml of 10 mM NH_4HCO_3 (pH 8.5), 400 mM NaCl.
11. Add equal volume scintillation liquid, mix well by vortexing and measure the signal using Liquid scintillation counter. The counting signal of [^{3}H], which is transformed from [^{3}H]-SAM to RNA substrates, represents the activity of tested MTases.

Recipes

1. 10x MTase Buffer
   0.5 M Tris-HCl (pH 7.5 or 8.0)
   50 mM KCl
   20 mM $MgCl_2$
   20 mM DTT
2. Cap-0 cap structure (m7GpppN-RNA)

Heat denatured RNA	13.5 μl

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10x Bodicon Capping Buffer	2 µl
10 mM GTP	2 µl
3 mM SAM	1 µl
RNase inhibitor 40 U/µl	0.5 µl
Bodicon Capping Enzyme (10 U/µl)	2 µl
Total	20 µl

3. Non-methylated Cap-0 cap structure (GpppN-RNA)

Heat denatured RNA	13.5 µl
10x Bodicon Capping Buffer	2 µl
10 mM GTP	2 µl
Inorganic pyrophospatase 0.1 U/µl	1 µl
RNase inhibitor 40 U/µl	0.5 µl
Bodicon Capping Enzyme (10 U/µl)	2 µl
Total	20 µl

4. MTase assay reaction mix

Purified MTases	1 µg
10x MTase Buffer	3 µl
GpppN-RNA or m7GpppN-RNA	2 µg
3 mM SAM	0.5 µl
[^{3}H]-SAM (67.3 Ci/mmol, 0.5 µCi/µl)	1 µl
RNase inhibitor 40 U/µl	1 µl
RNase free water	up to 30 µl
Total	30 µl

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