

Product Contents

Ribo m⁷G Cap Analog:

Part No.	Size (units)
P171A	10 A ₂₅₄ units
P171B	25 A ₂₅₄ units

Description: 5' 7-methyl guanosine nucleotide (m⁷G(5')ppp(5')G) or cap structure is incorporated into RNA synthesized in vitro to mimic the capped structure of mRNA. This product is intended for use with Riboprobe® and RiboMAX™ Systems.

Formula: C₂₁H₂₇N₁₀O₁₈P₃Na₂.

Formula Weight: 846.

Storage Conditions: See the product information label for storage recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

Volume: Part# P171A contains at least 13.7µl Ribo m⁷G Cap Analog in Nuclease-Free Water. Part# P171B contains at least 34.3µl Ribo m⁷G Cap Analog in Nuclease-Free Water.

Quality Control Assays

Activity Assays

Concentration: 40 ± 2mM, as determined by absorbance at 254nm.

Functional Assay: The Ribo m⁷G Cap Analog is tested for capped transcript synthesis using T7 RNA Polymerase and a 10:1 ratio of Ribo m⁷G Cap Analog to GTP. Capped and uncapped transcripts are separated by gel electrophoresis using a denaturing polyacrylamide gel. The minimum passing specification is > 50% capped transcripts.

References

1. Nakagawa, I. *et al.* (1980) A "capping" agent: P¹-S-Phenol P²-7-methylguanosine-5' pyrophosphorothioate. *Synthesis* 556.
2. Krieg, P.A. and Melton, D.A. (1987) In vitro RNA synthesis with SP6 RNA polymerase. *Meth. Enzymol.* **155**, 397–415.
3. Paterson, B.M. and Rosenburg, M. (1979) Efficient translation of prokaryotic mRNAs in a eukaryotic cell-free system requires addition of a cap structure. *Nature* **279**, 692–6.
4. Drummond, D.R., Armstrong, J. and Colman, A. (1985) The effect of capping and polyadenylation on the stability, movement and translation of synthetic messenger RNAs in *Xenopus oocytes*. *Nucl. Acids Res.* **13**, 7375–94.

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I. Standard Applications: Synthesis in vitro of Capped RNA Transcripts

Materials to Be Supplied by the User

All materials can be found in Section III.
(Solution compositions are provided in Section II.)

- DNA template, linearized
- Nuclease-Free Water
- Recombinant RNasin® Ribonuclease Inhibitor
- rNTP capping mix
- Transcription Optimized 5X Buffer
- Phage RNA Polymerase

1. In a microcentrifuge tube, add the following reagents at room temperature in the order listed:

Transcription Optimized 5X Buffer	10µl
DTT, 100mM	5µl
Recombinant RNasin® Ribonuclease Inhibitor	50 units
rNTP capping mix (see Section II)	5µl
Ribo m ⁷ G Cap Analog, 5mM	5µl
DNA template, linearized (in water or TE buffer)	5µg
Phage RNA Polymerase	40 units
Nuclease-Free Water to final volume of	50µl

2. Incubate for 1 hour at 37°C. To increase the yield of RNA, add an additional 40 units of Phage RNA Polymerase and incubate for 1 hour.

II. Composition of Buffers and Solutions

rNTP capping mix

5mM rATP
5mM rCTP
5mM rUTP
0.5mM rGTP
in Nuclease-Free Water

Transcription Optimized 5X Buffer

200mM Tris-HCl (pH 7.9 at 25°C)
50mM NaCl
30mM MgCl₂
10mM spermidine

5mM Ribo m⁷G Cap Analog

5mM Ribo m⁷G Cap Analog
in Nuclease-Free Water

III. Related Products

A. Related Systems

Product	Cat.#
Riboprobe® System—SP6	P1420
Riboprobe® System—T3*	P1430
Riboprobe® System—T7*	P1440
Riboprobe® System Buffers*	P1121
RiboMAX™ Large Scale RNA Production System—SP6*	P1280
RiboMAX™ Large Scale RNA Production System—T7*	P1300
TNT® SP6 Coupled Reticulocyte Lysate System	L4600
TNT® T3 Coupled Reticulocyte Lysate System	L4950
TNT® T7 Coupled Reticulocyte Lysate System	L4610
TNT® T7/SP6 Coupled Reticulocyte Lysate System	L5020
TNT® T7/T3 Coupled Reticulocyte Lysate System	L5010
TNT® SP6 Coupled Reticulocyte Lysate System, Trial Size	L4601
TNT® T7 Coupled Reticulocyte Lysate System, Trial Size	L4611
TNT® T7 Quick Coupled Transcription/Translation System	L1170
TNT® T7 Quick Coupled Transcription/Translation System, Trial Size*	L1171
TNT® SP6 Quick Coupled Transcription/Translation System	L2080
TNT® SP6 Quick Coupled Transcription/Translation System, Trial Size	L2081

*For Laboratory Use.

B. Related Products

Product	Size	Cat.#
SP6 Promoter Primer	2µg	Q5011
T3 Promoter Primer	2µg	Q5741
T7 Promoter Primer	2µg	Q5021
pGEM® Express Positive Control Template	10µg (2 × 5µg)	P2561
SP6 RNA Polymerase*	5,000u	P1081
	1,000u	P1085
SP6 RNA Polymerase (HC)*	2,500u	P4084
T7 RNA Polymerase*	5,000u	P2077
	1,000u	P2075
T7 RNA Polymerase (HC)*	10,000u	P4074
T3 RNA Polymerase*	1,000u	P2083
T3 RNA Polymerase (HC)*	2,500u	P4024
rATP, 10mM*	0.5ml	P1132
rCTP, 10mM*	0.5ml	P1142
rGTP, 10mM*	0.5ml	P1152
rUTP, 10mM*	0.5ml	P1162
Transcription Optimized 5X Buffer*	200µl	P1181
rATP, rCTP, rGTP and rUTP, each at 10mM*	0.5ml each	P1221
Nuclease-Free Water*	50ml (2 × 25ml)	P1193

*For Laboratory Use.

Product	Conc.	Size	Cat.#
Recombinant RNasin®			
Ribonuclease Inhibitor	20–40u/µl	2,500u	N2511
	20–40u/µl	10,000u	N2515

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