Certificate of Analysis

Ribo m^{7}G Cap Analog:

Part# 9PIP171

Revised 8/13

Part No.	Size (units)
P171A	10 A_{254} units
P171B	25 A_{254} units

Description: 5´ 7-methyl guanosine nucleotide (m^{7}G(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: $C_{21}H_{27}N_{10}O_{18}P_3Na_2$.

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 m^{7}G Cap Analog in Nuclease-Free Water. Part# P171B contains at least 34.3µl Ribo m^{7}G Cap Analog in Nuclease-Free Water.

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Quality Control Assays

Activity Assays

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

Functional Assay: The Ribo m^{7}G Cap Analog is tested for capped transcript synthesis using T7 RNA Polymerase and a 10:1 ratio of Ribo m^{7}G 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: P^1-S-Phenol P^2-7-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.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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Usage Information

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

Materials to Be Supplied by the User

All materials can be found in Section 3.
(Solution compositions are provided in Section 2.)

- 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 2)	5µl
Ribo m^7G 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.

2. 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 $MgCl_2$
10mM spermidine

5mM Ribo m^7G Cap Analog

5mM Ribo m^7G Cap Analog
in Nuclease-Free Water

3. 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

B. Related Products

Product	Size	Cat.#
SP6 Promoter Primer	2µg	Q5011
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

For Laboratory Use.

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