



Ribo m⁷G Cap Analog

Most eukaryotic mRNAs contain an m⁷G(5')ppp(5')G cap at the 5' end, which is important for the binding of translation initiation factors such as eIF4E, for RNA processing and transport, and for protection against cellular nucleases. The capped RNA can be used for *in vitro* translation with lysates, with *Xenopus* oocytes, and for processing and *in vitro* splicing in nuclear extracts (1,2). This Technically Speaking article answers questions on the role of the new Ribo m⁷G Cap Analog for *in vitro* transcription and translation.



What is Promega's Ribo m⁷G Cap Analog?

The Ribo m⁷G Cap Analog (Cat.# P1711, P1712) is a 5' 7-methyl guanosine nucleotide (m⁷G(5')ppp(5')G) that is incorporated into RNA synthesized *in vitro* to mimic the naturally occurring capped structure of mRNA. This cap structure is intended for use with Promega's Riboprobe^{®(a)} and RiboMAX^{TM(a,b,c)} Systems (1,2).



How is an m⁷G(5')ppp(5')G cap incorporated using the Riboprobe[®] System?

The Ribo m⁷G Cap Analog can be added directly to the *in vitro* transcription reaction at a 10:1 ratio of cap analog:rGTP (0.5mM cap analog:0.05mM rGTP). In the standard *in vitro* transcription reaction the concentration of rGTP is 0.5mM. When adding cap analog we recommend reducing the GTP to 0.05mM (1).



How is an m⁷G(5')ppp(5')G cap incorporated using the RiboMAXTM System?

The Ribo m⁷G Cap Analog can be added directly to the *in vitro* transcription at a 5:1 ratio of cap analog:rGTP (3mM cap analog:0.6mM rGTP). In the standard *in vitro* transcription reaction, the concentration of rGTP is 5mM for SP6, and 7.5mM for T3 and T7. When adding cap analog, we recommend reducing rGTP to 0.6mM (2).



What is the efficiency of incorporation of the Ribo m⁷G Cap Analog for *in vitro* transcription reactions?

The Ribo m⁷G Cap analog is incorporated as the initial 5' nucleotide in 60–70% of the transcripts under standard conditions using the Riboprobe[®] or RiboMAXTM Systems.



Is the yield of *in vitro* transcripts decreased when adding Ribo m⁷G Cap Analog to the standard reaction?

Incorporation of a cap analog reduces the yield of RNA to 20–50% of the standard reaction.

With long (>14kb) transcripts that are transcribed *in vitro* at a low rate or turn out to be smaller in size than expected, GTP may be limiting. Therefore, we recommend increasing the concentration of rGTP. The ratio of cap analog:rGTP can be varied from 10:1 to 1:1 to balance the percentage of capped products with the efficiency of transcription reaction. The reaction temperature can be decreased to 30°C to increase the length, but not the yield of the transcript.

With shorter transcripts, initiation might be limiting, thus we recommend increasing the incubation time, amount of RNA polymerase and concentration of template DNA.



Are all capped transcripts translated more efficiently than uncapped transcripts *in vitro* with lysates?

Many transcripts do not require a cap structure for efficient translation in the Rabbit Reticulocyte Lysate^(b,d) (RRL) or the Wheat Germ (WG) Systems (3,4,5). Comparable levels of protein synthesis can be achieved by increasing the amount of uncapped RNA added to the translation reaction (6).

In RRL, potassium chloride at 20mM above the maximal stimulatory level provides the optimal conditions for the synthesis from uncapped RNAs (7).

Translation in RRL is relatively independent of the presence of a cap structure on the mRNA. However, Svitkin *et al.* found that addition of several general RNA binding proteins (hnRNP A1, La, hnRNP 1/PTB, p50) render translation in RRL cap-dependent. These proteins drastically inhibit the translation of an uncapped mRNA, but have no effect on translation of a capped RNA (8). The authors suggest that one function of the general RNA binding proteins in the cytoplasm is to promote ribosome binding by a 5' end, cap-mediated mechanism, and thus prevent spurious initiations at aberrant translation start sites.

In RRL and WG translation systems, some capped transcripts may demonstrate increased translation efficiency (2,4,5). For example, Resto *et al.* (9) observed that for the *in vitro* translation of dihydrofolate reductase message in rabbit reticulocyte lysate, capped RNA yields 4–8X more protein than uncapped RNA.

Q Is the Flexi® Rabbit Reticulocyte Lysate System suitable for optimization with capped and uncapped transcripts?

Yes. The Flexi® Rabbit Reticulocyte Lysate System^(b,d) provides lysate devoid of added salts or DTT and provides potassium chloride, magnesium chloride and DTT for optimization of translation of uncapped or capped transcripts (10). Potassium chloride may be added to help improve fidelity of initiation from capped messages (7). The suggested concentration of KCl to add to the Flexi® Rabbit Reticulocyte Lysate System for capped transcripts is 70–100mM (1.4–2µl of 2.5M KCl stock per 50µl final reaction volume) (10).

Q Can cap analog be added directly to in vitro transcription/translation reactions (i.e., TNT® System reactions)?

Cap analog cannot be added directly to TNT® System^(b,d,e) reactions, as it dramatically inhibits translation. Also, unincorporated cap analog can inhibit translation if not removed from the capped mRNA reaction prior to translation.

REFERENCES

1. *Riboprobe® in vitro Transcription Systems Technical Manual #TM016*, Promega Corporation.
2. *RiboMAX™ Large Scale RNA Production Systems - SP6, T3, T7 Technical Bulletin #TB166*, Promega Corporation.

3. Dasso, M.C. and Jackson, J.J. (1989) *Nucl. Acids Res.* **17**, 3129.
4. *Rabbit Reticulocyte Lysate System Technical Manual #TM232*, Promega Corporation.
5. *Wheat Germ Extract Technical Manual #TM230*, Promega Corporation.
6. Gurevich, V.V. *et al.* (1991) *Anal. Biochem.* **195**, 207.
7. Kozak, M (1990) *Nucl. Acids Res.* **18**, 2828.
8. Svitkin, Y.V. *et al.* (1996) *EMBO J.* **15**, 7147.
9. Resto, E. *et al.* (1992) *Nucl. Acids Res.* **20** 5979.
10. *Flexi® Rabbit Reticulocyte Lysate System. Technical Bulletin #TB127*, Promega Corporation.

Flexi, *Riboprobe* and *TNT* are trademarks of Promega Corporation and are registered with the U.S. Patent and Trademark Office. *RiboMAX* is a trademark of Promega Corporation.

- ^(a)U.S. Pat. No. 5,552,302 and other patents. Inhibitors of Angiogenin, which comprises a segment of human PRI, is the subject of U.S. Pat. No. 4,966,964 and other patents assigned to the President and Fellows of Harvard College and exclusively licensed to Promega Corporation.
- ^(b)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.
- ^(c)The RiboMAX™ Large Scale RNA Production Systems-T7 and T3 (Cat.# P1290 and P1300) are covered by U.S. Pat. No. 5,256,555 and are sold under a license from Ambion, Inc. They are intended for research use only. Parties wishing to use these products for other applications should contact Ambion, Inc.
- ^(d)U.S. Pat. No. 5,283,179 and other patents. Certain applications of this product may require licenses from others.
- ^(e)U.S. Pat. Nos. 5,492,817, 5,665,563 and other patents.

