$m^7G(5')ppp(5')G$ Cap Analog

Store below –70°C.
Do not store in a frost-free freezer.

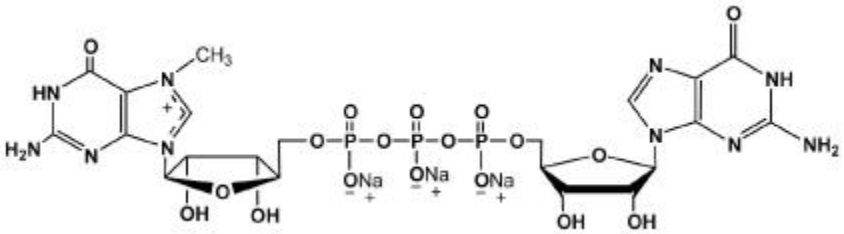
Catalog #:	AM8048	AM8050	AM8052
Amount (A_{254} U):	10 Units	25 Units	100 Units
Volume:	13.7 µL	34.25 µL	137 µL

Product Description: A cap analog designed for synthesis of 5' capped RNA molecules in in vitro transcription reactions.

Concentration: 40 mM (25 A_{254} = 1.37 x 10^{-6} mol = 1.16 mg)

Molecular Weight: 845 (Disodium salt)

Structure:



Storage Conditions: Store at or below –70°C. Avoid multiple freeze-thaw cycles. Aliquots of the product may be stored short-term at –20°C. ***Do not store in a frost-free freezer.***

Storage Buffer: Nuclease-free Water

USER INFORMATION

General Information: $m^7G(5')ppp(5)G$ (Cap Analog) is used for the synthesis of 5' capped RNA molecules in in vitro transcription reactions. Substitution of cap analog for a large portion of the GTP in an in vitro transcription reaction results in the incorporation of the cap structure into a large fraction of the transcripts. Capped mRNAs are generally translated more efficiently in reticulocyte and wheat germ in vitro translation systems. Uncapped mRNAs are rapidly degraded after microinjection into cells. Thus, it is important to cap in vitro transcripts for microinjection experiments.

Applications: For convenience, $m^7G(5')ppp(5')G$ is supplied at a concentration of 40 mM in water. If it is necessary to concentrate it, it may be lyophilized or dried in a SpeedVac® Concentrator without heat.

In vitro transcripts that will be microinjected into oocytes or other cells, used for transfection experiments, or used in in vitro splicing reactions, should be capped, because uncapped mRNAs are rapidly degraded after microinjection or transfection into cells. However, there are additional factors that should be considered:

- In vitro transcription reactions that contain high levels of cap analog produce significantly lower yields of transcript.
- Unincorporated cap analog is a potent inhibitor of protein synthesis initiation.

The standard strategy used to synthesize capped transcripts is to reduce the level of GTP to 10% of the normal concentration and replace the remaining 90% with cap analog [1]. This results in a high proportion of the transcripts being capped. Unfortunately, it also significantly decreases the yield of the transcription reaction, often to 20% or lower. To conserve cap analog and increase the yield of the transcription reaction, many scientists use a lower ratio of cap analog to GTP. A cap analog:GTP ratio of 4:1 is frequently used, but ratios as low as 1:1 are also used. It is likely, however, that the fraction of capped mRNA molecules decreases as the ratio of cap analog to GTP decreases. Thus, we recommend using a 4:1 ratio of Cap Analog to GTP as a reasonable compromise unless it is essential that a very high fraction of the transcripts be capped.

Cap Analog can be added to Ambion® MEGAscript® High Yield Transcription Kits (Cat #AM1330, AM1334, AM1338) to efficiently synthesize large amounts of capped RNA. The kit protocol and manual are available at **www.ambion.com/catalog/CatNum.php?1334**. For additional information on the procedure and protocol, please contact the Ambion Technical Services Department (1-800-888-8804, option 2 or TechServ@ambion.com).

Reference: Krieg PA and Melton DA (1987) In vitro RNA synthesis with SP6 RNA polymerase. *Methods in Enzymol* **155:** 397–415.

QUALITY CONTROL

Nonspecific Endonuclease Activity:	Meets or exceeds specification when a sample is incubated for 14–16 hr with 300 ng supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.
Exonuclease Activity:	Meets or exceeds specification when a sample is incubated for 14–16 hr with 40 ng ^{32}P-labeled *Sau*3A fragments of pUC19 and analyzed by PAGE.
RNase Activity:	Meets or exceeds specification when a sample is incubated for 14–16 hr with 25 ng ^{32}P-labeled RNA and analyzed by PAGE.
Functional Testing:	m^7G(5')ppp(5')G (Cap Analog) is analyzed for purity by HPLC and is shown to be ≥95% pure. Capping efficiency is tested using the MAXIscript® Kit (Cat #AM1312) and a gel shift assay. Cap Analog is tested in the MEGAscript® Kit (Cat #AM1334) for yield of capped RNA.

OTHER INFORMATION

Material Safety Data Sheets:

Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

Warranty and Liability:

For research use only. Not for use in diagnostic procedures.

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