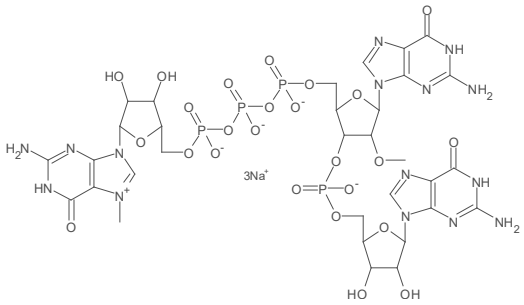
CleanCap® GG

CleanCap Reagent GG for Co-transcriptional Capping of mRNA
Catalog No. N-7133

Description

CleanCap Reagent GG is designed for the co-transcriptional capping of mRNA to produce an mRNA with naturally occurring Cap 1. Cap 1 mRNAs have superior in vivo activity compared to Cap 0 mRNA produced by legacy capping methods such as mCap or anti-reverse cap analog (ARCA). CleanCap can be used in conjunction with TriLink's catalog of modified NTPs.



CleanCap Reagent GG may be ordered using the following catalog numbers:
N-7133-1 (1 µmole)
N-7133-5 (5 µmole)
N-7133-10 (10 µmole)
For larger quantities, please call for a bulk quote.

Using the conditions described here, transcription with CleanCap GG and CleanCap GG (3' OMe) results in 70-90%[1] capped material, generating a Cap 1 structure and gives crude yields of ~1.5 mg per mL of transcription.

Use & Handling

50 mM in H_2O | Store at or below-20°C. | Upon first use, heat and prepare single use aliquots (see Protocol). | Use only certified RNase-free reagents and consumables with proper RNase-free technique.

QC Analysis

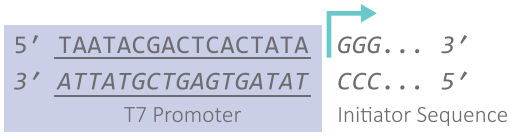
AX-HPLC	Mass Spec
^{31}P NMR	Conductivity
^{1}H NMR	

Product released by Quality Assurance.

[1]Final capping is dependent upon the CleanCap Reagent, DNA template and final mRNA sequence. Secondary structure due to RNA length and base composition can affect final capping efficiency.

Template Design

Template design is an integral part of any transcription. CleanCap GG is to be used with the initiating sequence 5’ GGG 3’. The figure below shows the correct T7 promoter sequence (underlined) and initiator sequence (italics) for CleanCap GG.



Customer Supplied Materials

NOTE: All reagents must be RNase free. Use recommended source or equivalent grade.

Required Reagents

- DNA Template
- Nucleoside-5’-Triphosphate (NTP) Set (TriLink cat. no. N-1505)
  Also available individually for use with modified NTPs. See Related Products for commonly used modified NTPs.
- T7 RNA polymerase (New England BioLabs cat. no. M0251S)
- Yeast Inorganic Pyrophosphatase (New England BioLabs cat. no. M2403S)
- Murine RNase Inhibitor (New England BioLabs cat. no. M0314S)
- 1M Tris-HCL (pH 8.0), RNase Free (Thermo Fisher Scientific cat. no. AM9856)
- Dithiothreitol (DTT) (EMD Millipore cat. no. 3860-5GM)
- Spermidine (Sigma Aldrich cat. no. 85558-1G)
- Triton X-100 (VWR cat. no. 80503-490)
- 1M Magnesium Acetate (Sigma Aldrich cat. no. 63052)
- UltraPure™ DNase/RNase-Free Distilled Water (Thermo Fisher Scientific cat. no. 10977015)

Optional Reagents

- RNaseZap™ RNase Decontamination Solution (Thermo Fisher Scientific cat. no. AM9780)

Protocol

RNase Free Techniques

It is essential that all reagents be rigorously RNase free. Use disposable RNase free tubes and bottles. Surfaces and pipettes can be wiped down with RNaseZap to destroy RNases. When possible, use dedicated RNase free pipettes. Avoid using pipettes that have been used for plasmid preparation using RNase A.

Preparation of Single Use Aliquots of CleanCap GG

1. Thaw CleanCap GG.
2. Heat 50 mM CleanCap GG for 15 min at 60°C.
3. Add DNase/RNase-Free water to required concentration.
   a. For TriLink recommended protocol add equal volume water for 25 mM.
4. Mix well by vortexing and aliquot into single use aliquots.
5. Use immediately or freeze at-20°C or below.

10X Transcription Buffer

400 mM Tris-HCL (pH 8)
100 mM DTT
20 mM Spermidine
0.02% Triton
165 mM Magnesium Acetate
DNase/RNase-Free Water

Protocol

Transcription Reaction

Add reagents in the proscribed order to ensure efficient transcription and capping.
Thaw reagents and store on ice.

1. Add RNase free water and NTPs to reaction tube.
2. Heat 25 mM CleanCap GG aliquot for 15 min at 60°C. Cool at room temperature for 5 min.
3. Immediately add CleanCap GG to tube and vortex to mix. Spin briefly to collect liquid.
4. Add 10X Transcription Buffer. Vortex.
5. Add DNA template.
6. Add Murine RNase Inhibitor, Yeast Inorganic Pyrophosphatase, and T7 RNA Polymerase.
7. Mix well by flicking or inverting tube 10 times and spin briefly to collect liquid.
8. Incubate at 37°C for 2-3 hours.

Table 2: Reaction Components

Component	**Final Concentration**	**100 µL Rxn**
DNase/RNase-Free Water	Up to 100 µL	Up to 100 µL
ATP (100 mM)	7.5 mM	7.5 µL
CTP[1] (100 mM)	7.5 mM	7.5 µL
GTP (100 mM)	1.5 mM	1.5 µL
UTP[1] (100 mM)	7.5 mM	7.5 µL
CleanCap GG (25 mM)	6 mM	24 µL
10X Transcription Buffer	1X	10 µL
DNA template	50 or 25 µg/mL[2]	5 µg or 2.5 ug[2]
Murine RNase Inhibitor (40 units/µL)	1 unit/µL	2.5 µL
Yeast Inorganic Pyrophosphatase (0.1 units/µL)	0.002 units/µL	2 µL
T7 RNA Polymerase (50 units/µL)	8 units/µL	16 µL
Total Volume	**100 µL**	**100 µL**

[1] Modified NTP can be used in place of wild-type. If using a modified NTP, use at the same concentration as the replaced wild-type NTP.
[2] Final Concentration of DNA template should be 50 µg/mL for a plasmid template or 25 µg/mL for a PCR template.

Related TriLink Products

Nucleoside-5'-Triphosphate (NTP) Set (cat. no. N-1505)
- Adenosine-5'-Triphosphate, ATP (cat. no. N-1510)
- Cytidine-5'-Triphosphate, CTP (cat. no. N-1511)
- Guanosine-5'-Triphosphate, GTP (cat. no. N-1512)
- Uridine-5'-Triphosphate, UTP (cat. no. N-1513)

5-Methylcytidine-5'-Triphosphate (cat. no. N-1014)
Pseudouridine-5'-Triphosphate (cat. no. N-1019)
N[1]-Methylpseudouridine-5'-Triphosphate (cat. no. N-1081)
5-Methoxyuridine-5'-Triphosphate (cat. no. N-1093)

TriLink offers several CleanCap derivatives. For optimal yield and capping, TriLink recommends using CleanCap AG whenever possible. CleanCap AG typically provides 94% capped material, and gives crude yields of 4 to 5 mg per mL of transcription. CleanCap GG results in 70-90% capped material and gives crude yields of ~1.5 mg per mL of transcription.
CleanCap Reagent AG (cat. no. N-7113)
CleanCap Reagent AG (3' OMe) (cat. no. N-7413)
CleanCap Reagent GG (3' OMe) (cat. no. N-7433)

Related TriLink Services

TriLink offers custom and stocked CleanCap Cap 1 mRNA in addition to the CleanCap Reagents. Please visit our website to learn more.

CleanCap® Products | RESEARCH LICENSE AGREEMENT

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