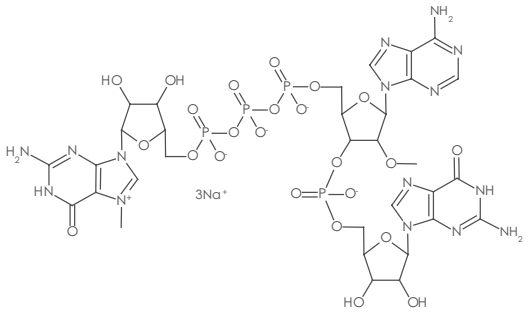
CleanCap® AG

CleanCap Reagent AG for Co-transcriptional Capping of mRNA
Catalog No. N-7113

Description

CleanCap Reagent AG 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 AG may be ordered using the following catalog numbers:
N-7113-1 (1 µmole)
N-7113-5 (5 µmole)
N-7113-10 (10 µmole)
For larger quantities, please visit our website for a bulk quote.

Using the conditions described here, transcription with CleanCap AG and CleanCap AG (3' OMe) results in 95%+[1] capped material, generating a Cap 1 structure and gives crude yields of 4 to 5 mg per mL of transcription.

Use & Handling

100 mM in H_2O | Store at or below-20°C. | Upon first use, prepare single use aliquots. | 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	

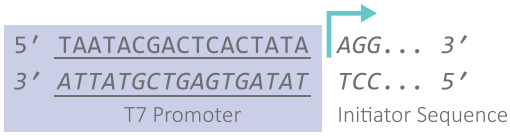
Product released by Quality Assurance.

[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.

Products containing CleanCap technology are for research use only. License is required for commercial use of CleanCap and CleanCap Products. For license restrictions and patent(s) information, refer to the Research License Agreement below.

Template Design

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



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.

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.
Store thawed enzymes on ice.

1. Add RNase free water and NTPs to reaction tube.
2. Add CleanCap AG to tube and vortex to mix. Spin briefly to collect liquid.
3. Add 10X Transcription Buffer. Vortex. Spin briefly to collect Liquid.
4. Add DNA template.
5. Add Murine RNase Inhibitor, Yeast Inorganic Pyrophosphatase, and T7 RNA Polymerase.
6. Mix well by flicking or inverting tube 10 times and spin briefly to collect liquid.
7. 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)	5 mM	5 µL
CTP[1] (100 mM)	5 mM	5 µL
GTP (100 mM)	5 mM	5 µL
UTP[1] (100 mM)	5 mM	5 µL
CleanCap AG (100 mM)	4 mM	4 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 or CleanCap AU whenever possible. CleanCap AG typically provides 95%+ 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 AU (cat. no. N-7114)
CleanCap Reagent AG (3' OMe) (cat. no. N-7413)
CleanCap Reagent GG (cat. no. N-7133)
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

PURCHASE AND/OR USE OF THIS PRODUCT SHALL CONSTITUTE ACKNOWLEDGMENT AND ACCEPTANCE OF THESE TERMS AND CONDITIONS.

Products containing the CleanCap technology (hereinafter "Products") and their use may be covered by one or more patents or pending Patent Applications. If Buyer does not agree to use the Products purchased pursuant to the terms and conditions set out in this Research License Agreement ("Agreement"), the Buyer should contact TriLink BioTechnologies, LLC within ten days of receipt to return the unused and unopened Products for a full refund; provided, however, that custom-made Products may not be returned for a refund.

1. Research Use. The purchase of Products containing CleanCap conveys to the buyer a non-exclusive, non-transferrable right to use the purchased amount of Products in internal research conducted by the buyer, whether the buyer is an academic, non-profit, or for-profit entity. Buyer agrees that it will not sell or otherwise transfer Products, or any components or derivatives thereof, to any third party. Notwithstanding the foregoing, materials made through use of the Products may be transferred by Buyer to Buyer's legal affiliates or bona fide third party contractors performing paid work on Buyer's behalf, provided the use by such third party contractors is limited to performance of work for Buyer and such work is performed subject to the terms of this Agreement.
2. Commercial Use. Buyer also agrees that it will not sell, transfer, or otherwise use Products, or any components or derivatives thereof, for any commercial purposes, including (a) any human, clinical or clinical trial use, including, without limitation, any administration into humans or any diagnostic or prognostic use; (b) any human germline modification, including modifying the DNA of human embryos or human reproductive cells; (c) any in vivo veterinary or livestock use; (d) the development, manufacture, distribution, importation, exportation, transportation, sale, offer for sale, marketing, promotion or other exploitation or use of the Patent Rights or a Product for or as a testing service, therapeutic or diagnostic for humans or animals; (e) Products that provide nutritional benefits and are regulated by a regulatory authority as a drug or biologic pursuant to Section 505 of the Federal Food, Drug, and Cosmetic Act of 1938, as amended, Section 351 of the Public Health Service Act of 1944, as amended, or any successor laws, or equivalent laws or regulations in jurisdictions outside the United States; (f) any agricultural use, including but not limited to the use or application in the cultivation, growth, manufacture, exportation, or production of any tobacco product; and (g) any use or application relating to gene drive, unless and until a license is obtained for such commercial use of Products, components, or derivatives thereof, regardless of the academic or non-profit status of the using entity. Information about commercial licenses for Products may be obtained by contacting TriLink BioTechnologies, LLC. Buyer may not use the Products to support the filing of a patent application that contains claims directed to the Products or uses thereof in any country in the world without the express approval of TriLink BioTechnologies, LLC.
3. Attribution. Buyers of the Products will expressly refer to the provision of the Products in their published and unpublished works by explicitly identifying the Products purchased and stating that the Products were "purchased from TriLink BioTechnologies, LLC (www.trilinkbiotech.com)."
4. Warranty. The Products are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, express or implied, and without any representation or warranty that the use or supply of the Products will not infringe any patent, copyright, trademark or other right. TriLink BioTechnologies, LLC does not recommend to its end users any particular application, methodology and/or protocol for the use of the Products. Depending on Buyer's particular use of the Products, it may be necessary to obtain a separate license or licenses from one or more third parties.
5. Limitation of Liability. TriLink BioTechnologies, LLC and its employees and agents shall not be held liable for your use of the Products transferred to you. Buyer agrees to hold TriLink BioTechnologies, LLC and its employees and agents harmless for any loss, claim, damage or liability, of whatsoever kind or nature, which may arise from acceptance, use, handling or storage of the Products by Buyer. In no event shall buyer be entitled to recover from TriLink BioTechnologies, LLC any special, indirect, incidental, consequential, or punitive damages in connection with this agreement, buyer's use of the Products, or the license granted hereunder.
6. Regulation Compliance. Upon receipt of Products, buyer shall use its expertise and facilities in strict compliance with all applicable local, state and federal laws, regulations and guidelines. Buyer understands that the Products may have biological and/or chemical properties that are unpredictable and unknown at the time of transfer, that they are to be used with caution and prudence, and that they will not to be used for testing in, or treatment of, humans.
7. Termination. Your right to have and use the Products will terminate immediately if Buyer fails to comply with the terms and conditions of this Agreement. Upon such termination of rights, Buyer shall destroy all Products, or any components or derivatives thereof, and notify TriLink BioTechnologies, LLC of such in writing.
8. Miscellaneous. This Agreement sets forth the complete and entire agreement of the Parties with respect to the subject matter hereof and supersedes and terminates all prior agreements and understandings between the Parties. No subsequent amendment or addition to this Agreement shall be binding upon the Parties unless reduced to writing and signed by the respective authorized officers of the Parties. This Agreement shall not be assigned or otherwise transferred by the buyer.