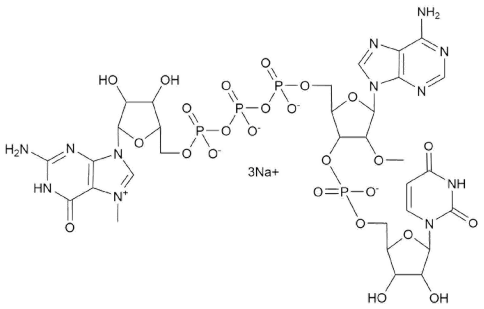
CleanCap® AU

CleanCap Reagent AU for Self-Amplifying mRNA
Catalog No. N-7114

Description

CleanCap Reagent AU is designed for the co-transcriptional capping of mRNA to produce an mRNA with naturally occurring Cap 1. CleanCap Reagent AU was specifically designed for self-replicating RNAs based on the genomes of positive sense (+) strand RNA viruses such as Venezuelan equine encephalitis virus (VEEV), Semliki forest virus (SFV) and Sindbis virus (SIN). The (+) strand genomes of these viruses start with a 5'-AU… Cap 1 mRNAs have superior in vivo activity compared to Cap 0 mRNA produced by legacy co-transcriptional capping methods anti-reverse cap analog (ARCA). CleanCap can be used in conjunction with TriLink's catalog of modified NTPs.



CleanCap Reagent AU may be ordered using the following catalog numbers:
N-7114-1 (1 µmole)
N-7114-5 (5 µmole)
N-7114-10 (10 µmole)
N-7114-100 (100 µmole)
For larger quantities, please visit our website for a bulk quote.

Using the conditions described here, transcription with CleanCap AU results in > 95%[1] capped material, generating a Cap 1 structure and crude yields of 4 mg to 6 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	Concentration
^{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. Not for use in diagnostic or therapeutic procedures. The purchase of this product conveys to the buyer the limited, non-transferable right to use the product only in internal research conducted by the buyer as defined in the Research License Agreement.

Template Design

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

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)
- Inorganic Pyrophosphatase (New England BioLabs cat. no. M0361S or 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
270 mM Magnesium Acetate
DNase/RNase-Free Water

Protocol

Transcription Reaction

Add reagents in the prescribed 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 AU 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 AU (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 structures. CleanCap AG and CleanCap AG (3' OMe) typically provide > 95% capped material, and crude yields of 4 mg to 5 mg per mL of transcription.

CleanCap Reagent AG (cat. no. N-7113)
CleanCap Reagent AG (3' OMe) (cat. no. N-7413)

Related TriLink Services

TriLink offers custom 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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