

Product Data Sheet

Product Name: CleanCap Reagent AU

Cat. No.: GN20150

Chemical Properties

Purity	>95.00%	Extinction Coefficient	33,400 Lmol ⁻¹ cm ⁻¹ at 258 nm
Formula	C ₃₁ H ₄₂ N ₁₂ O ₂₅ P ₄ (free acid)	M.Wt	1106.6 g/mole (free acid)
Salt Form	Na ⁺	Concentration	102mM
Buffer	H ₂ O	Storage	-20°C or below, always avoid freeze-thaw cycles.
Synonyms	CleanCap AU for Self-Amplifying mRNA, CleanCap Reagent AU for co-transcriptional capping of mRNA, m ⁷ G(5')ppp(5') (2'OMeA)pU	Backbone	5'-5'-Triphosphate
Base Analog	Adenosine, Uridine	Sugar Type	RNA
Nucleotide Category	Cap analogs		
Applications	CRISPR, In vitro Transcription, Recombinases, TALENS, Transposases, Vaccine Development, Zinc Finger Nucleases		

Protocol

This protocol only provides a guide, please modify it to meet your specific needs.

CleanCap Reagent AU is used for transcription reactions:

1. Add RNase-free water and NTPs to the reaction tube.
2. Add CleanCap Reagent AU to the tube and vortex to mix. Briefly centrifuge to collect the liquid at the bottom of the tube.
3. Add 10X transcription buffer. Vortex to mix. Briefly centrifuge to collect the liquid at the bottom of the tube.

Caution: Product has not been fully validated for medical applications. For research use only.

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4. Add DNA template.
5. Add murine RNase inhibitor, yeast inorganic pyrophosphatase, and T7 RNA polymerase.
6. Gently flick or invert the tube 10 times to mix thoroughly, then briefly centrifuge to collect the liquid at the bottom of the tube.
7. Incubate at 37°C for 2-3h.

Note: All reagents must be strictly RNase-free. Use disposable RNase-free tubes and bottles. Surfaces and pipettes can be wiped with RNaseZap to inactivate RNase. If possible, use dedicated RNase-free pipettes. Avoid using pipettes that have been used for RNase A plasmid preparation.

Background

CleanCap Reagent AU is specifically designed for co-transcriptional capping, with the structure m⁷G(5')ppp(5')(2'OMeA)pU, to produce mRNA with a natural Cap1 structure^[1]. CleanCap Reagent AU is specifically designed for self-replicating RNA based on the genomes of positive-strand RNA viruses (such as Venezuelan equine encephalitis virus (VEEV), Semliki Forest virus (SFV), and Sindbis virus (SIN)), whose positive-strand genomes begin with 5'-AU...^[2, 3]. Compared to Cap0 mRNA prepared by traditional co-transcriptional capping methods (such as anti-reverse cap analog, ARCA), mRNA with a Cap1 structure exhibits superior activity, stability, and lower immunogenicity in vivo^[4]. CleanCap Reagent AU can be used in conjunction with GlpBio's series of modified NTP products.

References:

- [1] Henderson J M, Ujita A, Hill E, et al. Cap 1 messenger RNA synthesis with co-transcriptional cleancap® analog by in vitro transcription[J]. Current protocols, 2021, 1(2): e39.
- [2] Lu X, Wu Y, Zhao C, et al. Co-expression of HSV-1 ICP34. 5 enhances the expression of gene delivered by self-amplifying RNA and mitigates its immunogenicity[J]. FEBS Open Bio, 2025.
- [3] Kairuz D, Samudh N, Ely A, et al. Production, characterization, and assessment of permanently cationic and ionizable lipid nanoparticles for use in the delivery of self-amplifying RNA vaccines[J]. Pharmaceutics, 2023, 15(4): 1173.
- [4] Zhang H, Ma J, Ma T, et al. GPT-driven generation and biological activity evaluation of novel mRNA trinucleotide Cap1 analogs for mRNA vaccine or immunotherapy[J]. Journal of Materials Chemistry B, 2025.

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