

## Description

TriLink's CleanCap® AG (3' OMe) CleanScript™ IVT Kit provides essential components for mRNA synthesis by *in vitro* transcription (IVT) with co-transcriptional capping. The kit components are sufficient to run 25 x 100 µL or 125 x 20 µL reactions following our recommended IVT protocol. It includes a cap analog, modified and unmodified nucleotides, IVT enzymes, a reaction buffer, and a control DNA template. Each kit is expected to yield 20-25 mg of capped mRNA.

The kit is designed for:

- **Time and cost savings** – Co-transcriptional capping protocol with all necessary reagents for a single-tube reaction providing high yield, fewer steps, and less handling
- **High capping efficiency** – Protocol with CleanCap® AG (3' OMe) cap analog to produce an optimal cap 1 with over 95% capping efficiency\*\*
- **Increased mRNA yield** – Buffer and kit components formulated using TriLink's proprietary CleanScript® IVT method to yield up to 1 mg capped mRNA per 100 µL reaction from 5 µg linear plasmid
- **Ultra-low double-stranded RNA (dsRNA)** – Up to 85% dsRNA reduction during IVT using a combined CleanScribe™ RNA Polymerase and CleanScript method instead of wild-type T7 RNA polymerase in a standard method
- **Enhanced mRNA performance** – Robust protein expression and low inflammatory responses from mRNA synthesized with CleanCap AG (3' OMe), CleanScript method, CleanScribe RNA polymerase, and optional N1-methylpseudouridine

## Use & handling

Store at -15 to -20 °C. Each kit contains sufficient reagents for 25 x 100 µL or 125 x 20 µL reactions. Use only certified RNase-free reagents and consumables with proper RNase-free technique.

**Table 1: Reaction components of the kit**

| Component                               | Cap color | Concentration | Amount   |
|---|-----------|---------------|----------|
| CleanCap® Reagent AG (3' OMe)           | ● blue    | 100 mM        | 10 µmol  |
| Adenosine-5'-Triphosphate               | ○ clear   | 100 mM        | 100 µmol |
| Cytidine-5'-Triphosphate                | ○ clear   | 100 mM        | 100 µmol |
| Guanosine-5'-Triphosphate               | ○ clear   | 100 mM        | 100 µmol |
| Uridine-5'-Triphosphate                 | ○ clear   | 100 mM        | 100 µmol |
| N1-Methyl-Pseudouridine-5'-Triphosphate | ● green   | 100 mM        | 100 µmol |
| AG CleanScribe™ RNA Polymerase Mix      | ● orange  | 10X           | 250 µL   |
| 10X AG CleanScript™ IVT Buffer          | ● yellow  | 10X           | 1 mL     |
| FLuc Control Plasmid                    | ● red     | 0.5 µg/µL     | 25 µg    |



**Figure 1. CleanCap® AG (3' OMe) CleanScript™ IVT Kit**

## QC analysis

Each kit component is tested to meet our standard QC specifications. Each kit lot is functionally verified to yield a 1.9 kb FLuc mRNA following our recommended protocol with the included control.

Product released by Quality Assurance. TriLink is certified ISO 9001:2015 for kit components, and Alphazyme is certified ISO 13485:2016 for enzyme manufacturing.

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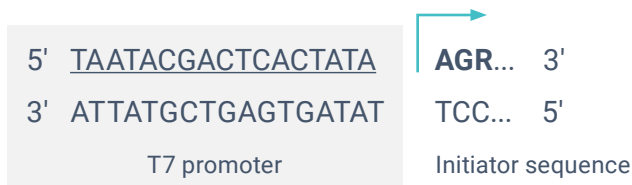
\*\*Final capping is dependent upon the CleanCap Reagent, DNA template, and final mRNA sequence. Secondary structure due to RNA length and base composition can affect final capping efficiency, mRNA yield, and translation efficiency.

## Important notes

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### Template design

Template design is an integral part of *in vitro* transcription. Template can be linearized plasmid DNA or PCR products. CleanCap AG (3' OMe) is designed to be used with an initiating sequence of 5' AGG 3' or 5' AGA 3'. The figure below shows the correct T7 promoter sequence (underlined) and initiator sequence (bold where R is G or A) for CleanCap AG (3' OMe).



For downstream applications that require translated mRNA, ensure that the DNA template encodes the necessary mRNA features. Most translated mRNAs contain a cap-1 structure provided by CleanCap AG (3' OMe), a 5' UTR, a protein coding sequence, a 3' UTR, and a poly(A) tail. Alternatively, poly(A) tailing can also be performed post-transcriptionally with a poly(A) polymerase (not included in the kit).

### Template quality

The efficiency and yield of the transcription reaction are significantly affected by the quality of the DNA template. Linearized plasmid DNA must be fully digested as residual circularized plasmid will produce extended RNA byproducts. Templates must also be devoid of contaminants like RNase, protein, and salts. Using restriction enzymes that produce blunt ends or 5'-overhangs is recommended, along with phenol/chloroform or silica-membrane purification. While PCR mixtures can be employed directly, using silica-membrane-purified PCR products generally enhances the results.

### Customer supplied materials

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

#### Required reagents

- DNase/RNase-free water
- DNA template (see above for design considerations)

#### Optional reagents

- DNase I (RNase-free) (New England BioLabs cat. no. M0303S)
- CaCl<sub>2</sub> (Calcium chloride solution, BioUltra, 1M) (Sigma-Aldrich cat. no. 21115)
- RNeasy Kits (QIAGEN cat. no. 74104 or 75144) or alternative purification method
- RNaseZap™ RNase Decontamination Solution (Thermo Fisher Scientific cat. no. AM9780)

### Protocol for CleanCap® AG (3' OMe) CleanScript™ IVT Kit

[trilinkbiotech.com/cleancap-ag-3-ome-ivt-kit.html](http://trilinkbiotech.com/cleancap-ag-3-ome-ivt-kit.html)

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#### RNase-free techniques

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

#### Reagent preparation

Add reagents in the prescribed order to help ensure efficient transcription and capping. Ensure each component is homogenous before use. Store enzyme mix on ice. These reaction conditions have been tested with templates up to 6 kb in length.

## Reaction setup

If performing a 20  $\mu$ L 100  $\mu$ L IVT reaction, use the volumes listed in Table 2. If using a different volume, adjust accordingly. To enable tracking during reaction setup, each kit component is color-coded in the instruction to its corresponding cap.

- Place the CleanScribe RNA Polymerase Mix (● orange cap) on ice.
- Thaw remaining components at room temperature. White precipitates may form in the buffer during storage but will not affect IVT performance. Vortex or mix the solution thoroughly to redissolve the precipitates before use.
  - Vortex them briefly to mix.
  - Pulse-spin to collect liquid.
- Combine the water<sup>1</sup> and the CleanScript IVT buffer (● yellow cap) in a clean microcentrifuge or PCR tube. Limit air exposure to the buffer, which contains DTT, to prevent oxidation and maintain IVT performance.
  - Vortex it briefly to mix.
  - Pulse-spin to collect liquid.
- Add NTPs (○ clear cap for unmodified, ● green cap for N1 $\Psi$ ), CleanCap AG (3' OMe) (● blue cap), and DNA template (● red cap for FLuc control) to the reaction tube.
  - Vortex it briefly to mix.
  - Pulse-spin to collect liquid.
- Add the CleanScribe RNA Polymerase Mix (● orange cap) to the reaction tube.
  - Vortex it briefly to mix or flick it gently to mix.
  - Pulse-spin down briefly.
- Incubate at 37 °C for 3 hours. We recommend a thermal cycler with heated lid or dry air incubator to avoid evaporation and condensation on the lid.

**Table 2: Reaction setup.** If using a different reaction volume, adjust accordingly.

| Protocol step | Component   | Final concentration            | Amount per 20 $\mu$ L rxn   | Amount per 100 $\mu$ L rxn   |
|---------------|---|--------------------------------|-----------------------------|------------------------------|
| 3             | DNase/RNase-free water <sup>1</sup>                                   | N/A                            | Up to 7.2 $\mu$ L           | Up to 36 $\mu$ L             |
|               | 10X AG CleanScript™ IVT Buffer <sup>2</sup>                           | 1X                             | 2 $\mu$ L                   | 10 $\mu$ L                   |
| 4             | ATP (100 mM)  | 10 mM                          | 2 $\mu$ L                   | 10 $\mu$ L                   |
|               | CTP (100 mM)  | 10 mM                          | 2 $\mu$ L                   | 10 $\mu$ L                   |
|               | GTP (100 mM)  | 10 mM                          | 2 $\mu$ L                   | 10 $\mu$ L                   |
|               | UTP (100 mM) or N1 $\Psi$ (100 mM) <sup>3</sup>                       | 10 mM                          | 2 $\mu$ L                   | 10 $\mu$ L                   |
|               | CleanCap® AG (3' OMe) (100 mM)  | 4 mM                           | 0.8 $\mu$ L                 | 4 $\mu$ L                    |
|               | DNA template <sup>4</sup><br>Linearized plasmid<br>OR<br>PCR template | 50 $\mu$ g/mL<br>25 $\mu$ g/mL | 1 $\mu$ g<br>0.5 $\mu$ g    | 5 $\mu$ g<br>2.5 $\mu$ g     |
| 5             | AG CleanScribe™ RNA Polymerase Mix (10X)                              | 1X                             | 2 $\mu$ L                   | 10 $\mu$ L                   |
|               | <b>Total Volume</b>   | <b>N/A</b>                     | <b>20 <math>\mu</math>L</b> | <b>100 <math>\mu</math>L</b> |
| 6             | <b>Incubate at 37 °C, 3 hours</b>                                     |                                |                             |                              |

<sup>1</sup> Calculate  $\mu$ L of water to add by subtracting the DNA template volume from the specified "up to" value.

<sup>2</sup> 10X AG CleanScript IVT Buffer contains DTT. Prolonged air exposure may oxidize the DTT and reduce IVT performance.

<sup>3</sup> N1-methylpseudouridine (N1 $\Psi$ ) can be used in place of uridine (UTP) for potentially reduced inflammatory responses. If using N1 $\Psi$ , replace UTP with the same concentration of N1 $\Psi$ .

<sup>4</sup> Final concentration of DNA template should be 50  $\mu$ g/mL for a plasmid template or 25  $\mu$ g/mL for a PCR template. When using TriLink's FLuc control template, add 2  $\mu$ L of the 0.5  $\mu$ g/ $\mu$ L linearized plasmid provided for 1  $\mu$ g total in a 20- $\mu$ L IVT or 10  $\mu$ L for 5  $\mu$ g total in a 100- $\mu$ L IVT.

## Post-transcriptional options

### DNase treatment

DNase treatment may be performed according to the recommended one-pot IVT/DNase reaction below or according to a vendor-recommended protocol. If following a vendor-recommended protocol, we recommend performing an initial purification (RNeasy kit or equivalent) before the DNase treatment followed by a second RNA clean up post-DNase treatment. The pre-DNase cleanup reduces the risk of any potential DNase incompatibility with the CleanScript IVT buffer.

For best one-pot DNase results, add the reagents as described in Table 3 to the final 100  $\mu$ L IVT reaction tube. Mix by inverting or gently flicking the tube and pulse-spin to collect liquid. Incubate at 37 °C for 1 hour then proceed to purification.

**Table 3: Example CleanScript IVT DNase reaction for 100  $\mu$ L IVT.** Adjust volumes or working concentrations of the components accordingly for a different IVT volume.

| Component                  | Final concentration | Volume                         |
|----------------------------|---------------------|--------------------------------|
| IVT reaction               | N/A                 | 100 $\mu$ L                    |
| Water                      | N/A                 | 7.67 $\mu$ L                   |
| CaCl <sub>2</sub> (200 mM) | 3.5 mM              | 2.33 $\mu$ L                   |
| DNase (2 U/ $\mu$ L)       | 0.35 U/ $\mu$ L     | 23.3 $\mu$ L                   |
| <b>Total</b>               | <b>N/A</b>          | <b>133.3 <math>\mu</math>L</b> |

### Purifications

IVT reactions may be purified by traditional methods such as lithium chloride precipitation or spin columns (for example, QIAGEN RNeasy mini or midi kit) for higher purity at small scales. A typical CleanScript IVT following the kit protocol yields 0.8-1 mg of RNA per 100  $\mu$ L of reaction using unmodified NTPs or N1 $\Psi$ . Note that recovery of your mRNA from the purification process may vary.

## Frequently asked questions

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### General

**Q:** How can I prevent RNase contamination?

**A:** Use only certified RNase-free reagents and consumables, clean surfaces and pipettes with the RNaseZap solution.

**Q:** Can I use the reaction buffer, enzyme mix, and protocol of my kit for other caps?

**A:** They are specifically formulated for their corresponding kit and should not be used with another kit or cap for optimal results.

### Template

**Q:** Can I use circular plasmid DNA as a template?

**A:** No, the plasmid DNA must be fully linearized. Residual circular plasmid will produce unwanted extended RNA byproducts that can affect your results.

**Q:** Can I use PCR products directly in the reaction?

**A:** Yes, PCR products can be used directly, though using silica-membrane-purified PCR products typically gives better results. The final concentration should be 25 µg/mL for PCR templates (compared to 50 µg/mL for plasmid templates).

**Q:** What's the minimum sequence requirement for proper capping?

**A:** The template must contain the correct T7 promoter sequence followed by an initiating sequence of 5' AGG 3' or 5' AGA 3' for proper use with CleanCap AG (3' OMe).

### Control template

**Q:** Why is the FLuc control template provided?

**A:** The control template is included to verify RNA transcription and aid with troubleshooting of technical issues that might arise. Following our [recommended protocol](#), the template should yield 0.8-1 mg of 1.9 kb RNA transcript in 3 hrs of IVT.

**Q:** Do I need to linearize the FLuc control template?

**A:** No, the FLuc plasmid template is provided linearized.

**Q:** How much FLuc control template is provided?

**A:** 25 µg of linearized control template is provided. The amount is sufficient to use as a control for 5 x 100 µL reactions.

**Q:** What is the poly(A) tail length in the FLuc control plasmid?

**A:** It is 80A.

### IVT

**Q:** What is in the CleanScribe™ RNA Polymerase Mix?

**A:** It contains CleanScribe™ RNA Polymerase, RNase inhibitor, and inorganic pyrophosphatase.

**Q:** What size templates can I use with this kit?

**A:** The reaction conditions have been tested with templates up to 6 kb in length.

**Q:** How do I prepare all the reagents prior to IVT set-up?

**A:** It is best to set up the reaction at room temperature. Thaw all the components except the enzyme mix at room temperature. Mix components by vortexing briefly, pipetting up and down a few times, or flicking gently; then spin down. Keep the enzyme mix on ice.

**Q:** What is the expected yield from a standard reaction?

**A:** A typical CleanScript IVT reaction following the [kit protocol](#) yields 0.8-1 mg of RNA per 100 µL of reaction using unmodified NTPs or N1Ψ.

**Q:** How long should I incubate the IVT reaction?

**A:** The standard protocol calls for a 3-hour incubation at 37°C, using either a thermal cycler with heated lid or a dry air incubator to prevent evaporation and condensation.

**Q:** Can I use your kits to scale my reaction above 100 µL?

**A:** It is possible by adjusting the component amounts proportionally. Note that you may need to optimize reaction conditions to increase mRNA yield linearly while maintaining quality.

**Q:** Can I use your kit protocol to make uncapped mRNA?

**A:** With our CleanCap® AG (3' OMe) and CleanCap® M6 IVT kits for mRNA, you may omit the cap in our published protocols to obtain uncapped mRNA. A similar yield is expected. However, if your template is 'AG start', there will be more heterogeneity at the 5' end, for example a mix of A and G. Depending on the required application of the uncapped mRNA, phosphatase treatment can be used to remove the 5' triphosphate if required.

## Purification

**Q:** Do I need to perform DNase treatment?

**A:** While DNase treatment is optional, it is recommended to remove template DNA. You can either use the one-pot DNase protocol [provided above](#) or purify the RNA and then follow a DNase treatment.

**Q:** What purification methods are recommended after the IVT reaction?

**A:** You can use lithium chloride precipitation, spin columns like QIAGEN RNeasy kits, or oligo(dT) if your RNA has a poly(A) tail. The choice often depends on your scale and downstream applications.

## Troubleshooting

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**Q:** I did not get any mRNA using the kit. What should I do?

**A:** First, confirm all components were added to the IVT tube. Check the integrity of your DNA template (linearized plasmid or PCR fragment) on a gel. Run a control IVT reaction with the included FLuc control plasmid alongside your own template for troubleshooting. Also ensure that your DNA template sequence contains a T7 promoter and AG start site.

**Q:** I saw a large smear with low yield of my sample after IVT. What happened?

**A:** In most cases, a large smear with low yield indicates mRNA degradation from RNase contamination or poor template quality. While all kit components are free of RNase and DNase, please ensure the reaction is set up in an RNase-free environment (benchtop, pipet tips, containers, etc). Follow practices that minimize RNase contamination with tube handling and reaction setup.

If there is no RNase contamination, other issues could be with template quality and template sequence. Follow practices that reduce DNase contamination, such as working in a DNase-free area when setting up the IVT reaction and using DNase in a designated area post-IVT. It is important to check the quality of the linearized DNA template for a single clean band.

**Q:** I got a low yield using CleanCap® AG (3' OMe) IVT kit. What can I do to increase the yield?

**A:** The [CleanScript protocol](#) of the kit typically results in 0.8-1 mg of RNA per 100 µL of reaction. If your yield is lower, run a control reaction with the included FLuc control plasmid alongside your own template for troubleshooting.

**Q:** How can I optimize 5' UTR and 3' UTR sequences?

**A:** Optimization of the 5' and 3' untranslated regions (UTRs) depends on your specific goals and the target cell or tissue type for your mRNA.

To support this, our [Officinae Bio services](#) offer expert optimization services. Send us your sequence and UTRs, and we'll perform codon optimization to help ensure optimal expression.

Additionally, reviewing published literature can be a valuable starting point for identifying UTR sequences relevant to your application. We also recommend conducting screening experiments to evaluate translational efficiency.

If you are using [TriLink's mRNA services](#), we have our own UTR sequences integrated in the DNA template as an option to insert your open reading frame (ORF) for a streamlined workflow.

## Product support

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For additional questions or technical support around this product, please contact [support@trilinkbiotech.com](mailto:support@trilinkbiotech.com)

## Related TriLink products

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### Kits

CleanCap<sup>®</sup> M6 IVT Kit, High Yield (cat no. K-7453)

### Enzymes

CleanScribe<sup>™</sup> RNA Polymerase (cat. no. E-0107)

T7 RNA Polymerase (cat no. E-0053)

Inorganic Pyrophosphatase (cat no. E-0051)

Engineered RNase Inhibitor (cat no. E-0075)

BspQI (cat no. E-0097)

### Nucleotides

Adenosine-5'-Triphosphate, ATP (cat no. N-1501)

Cytidine-5'-Triphosphate, CTP (cat no. N-1502)

Guanosine-5'-Triphosphate, GTP (cat no. N-1503)

Uridine-5'-Triphosphate, UTP (cat no. N-1504)

N1-Methylpseudouridine-5'-Triphosphate (cat no. N-1081)\*

rNTP Set: ATP, GTP, CTP, UTP (cat no. N-1505)

rNTP Set with N1MePsUTP (cat no. N-1506)\*

### Cap analogs

CleanCap<sup>®</sup> Reagent M6 (cat no. N-7453)

CleanCap<sup>®</sup> Reagent AG (3' OMe) (cat no. N-7413)

CleanCap<sup>®</sup> Reagent AG (cat no. N-7113)

CleanCap<sup>®</sup> Reagent AU (cat no. N-7114)

### Ready-made mRNAs

CleanCap<sup>®</sup> M6 EGFP mRNA (N1MePsU) (cat no. L-8101)<sup>‡</sup>

CleanCap<sup>®</sup> M6 FLuc mRNA (N1MePsU) (cat no. L-8102)

CleanCap<sup>®</sup> M6 mCherry mRNA (N1MePsU) (cat no. L-8103)

CleanCap<sup>®</sup> M6 Cas9 mRNA (N1MePsU) (cat no. L-8106)<sup>§</sup>

CleanCap<sup>®</sup> M6 EPO mRNA (N1MePsU) (cat no. L-8109)

CleanCap<sup>®</sup> M6 Cre mRNA (N1MePsU) (cat no. L-8111)

CleanCap<sup>®</sup> M6 EGFP ModTail<sup>™</sup> mRNA (N1MePsU) (cat no. L-8101A)<sup>‡</sup>

CleanCap<sup>®</sup> M6 FLuc ModTail<sup>™</sup> mRNA (N1MePsU) (cat no. L-8102A)

CleanCap<sup>®</sup> M6 Cas9 ModTail<sup>™</sup> mRNA (N1MePsU) (cat no. L-8106A)<sup>§</sup>

## Related TriLink services

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TriLink offers RUO and GMP custom mRNA production services in addition to our catalog offerings. Visit our website [trilinkbiotech.com/mrna-cdmo-services](https://trilinkbiotech.com/mrna-cdmo-services) or contact us at [mrna-services@trilinkbiotech.com](mailto:mrna-services@trilinkbiotech.com) for more information.

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