



REFERENCE GUIDE

Enhance your RNA performance
with modified and unmodified
nucleotides



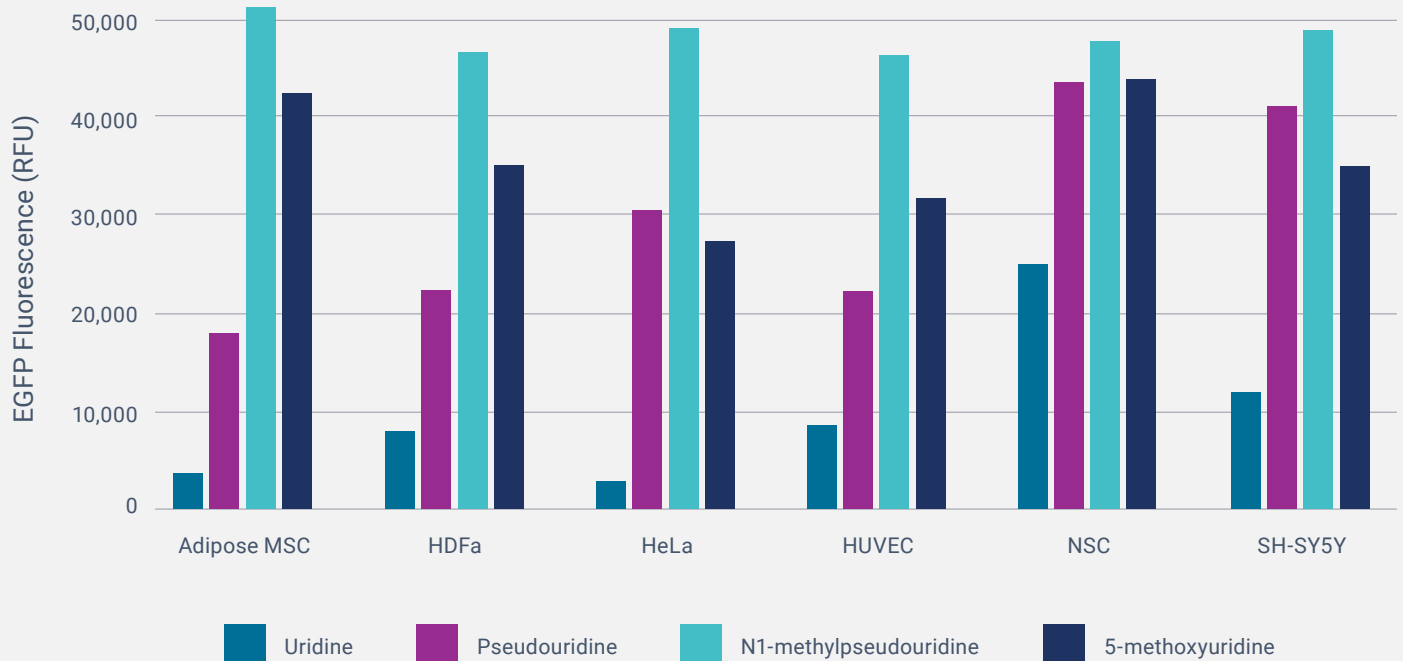


A proven solution for mRNA therapeutic development

The use of modified nucleoside triphosphates (NTPs) has been instrumental in advancing mRNA therapeutic and vaccine innovation, and TriLink BioTechnologies® has been partnering with leading pharma and biotech pioneers since day one. From research use to the first-ever GMP-grade offering, TriLink provides a comprehensive portfolio of off-the-shelf, scalable, chemically synthesized NTPs to help accelerate your mRNA therapeutic or vaccine development.

Incorporating modified uridines into mRNA products reduces immunogenicity, enhances mRNA stability, and increases translational capacity for more successful therapeutic action.

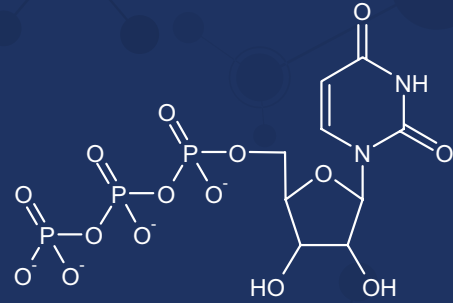
Figure 1. Improved mRNA activity with modified uridines



Four capped mRNA constructs coding for the enhanced green fluorescent protein (EGFP) were transcribed with different uridines: unmodified uridines (U), pseudouridine (Ψ), N1-methylpseudouridine (N1meΨ), 5-methoxyuridine (5-methoxyU). 20 ng of each respective mRNA was transfected into the following cell lines: adipose mesenchymal stem cells (MSC), dermal fibroblasts cells (HDFa), cervical cell line HeLa, umbilical vein endothelial cells (HUVEC), human neural stem cells (NSC), and neuroblastoma line SH-SY5Y. GFP fluorescence was measured after 45 hours at 535 nm. Values are reported as relative fluorescence units (RFU) from background. All modified mRNAs produced more EGFP signal than the construct with unmodified uridines, and the construct with N1meΨ produced the highest signal.

>150 NTP types to meet diverse research needs:

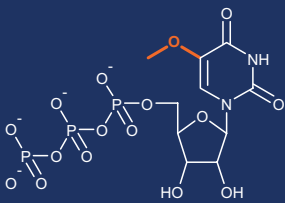
Unmodified bases



Uridine-5'-triphosphate

We are available to provide your desired modifications on nucleotides of your choice for mRNA synthesis and beyond. Our off-the-shelf modified uridines include:

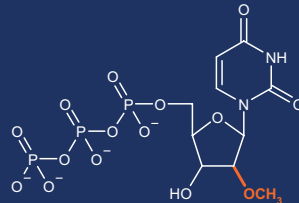
Base modified



5-methoxyuridine-5'-triphosphate

Modifying the nucleobase can improve mRNA performance by masking therapeutic mRNA from the immune responses to exogenous mRNAs.

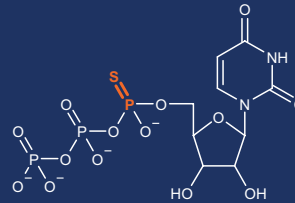
Sugar modified



2'-O-methyluridine-5'-triphosphate

Modifying the ribose sugar at 2' hydroxyl position (e.g., 2'-O-methyl) can impart RNA nuclease resistance. Sugar modifications can impact the yield of in vitro transcription reactions and may require optimization.

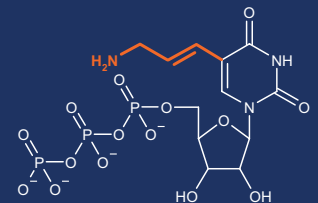
Alpha phosphate modified



Uridine-5'-O-(1-thiotriphosphate)

Adding a thiophosphate at the NTP's alpha position allows for a modified phosphorothioate backbone, accelerating the rate of translation initiation. Adding 1-thio-ATP for the polyadenylation step can prevent degradation of the poly(A) by 3' deadenylases.

Linker modified



5-aminoallyluridine-5'-triphosphate

Specific base modifications (like aminoallyl) provide a primary amine for easy coupling with molecules of interest (biotin, fluorescent dyes, etc.). These modified RNAs are useful in discovery settings or for RNA enrichment.

Consider CleanCap® capping technology to reduce overall cost and time in mRNA production



In conjunction with the modified nucleotides, using a CleanCap® analog further enhances mRNA's protein expression. Our latest CleanCap® M6 analog produces mRNA more

potent than enzymatically capped constructs and outperforms previous CleanCap® analogs.

Due to the co-transcriptional capping process and Cap 1 structure, the CleanCap analogs also help reduce the cost and time of manufacturing mRNA constructs. Co-transcriptional capping allows *in vitro* transcription and capping in one pot, requiring only one purification and bioreactor step without the need for additional reagents and enzymes, thereby streamlining your manufacturing process.

Learn more about CleanCap technology at trilinkbiotech.com/cleancap



ISO 9001:2015 certified manufacturing facilities



High quality standards on RUO and GMP products and services



Simplified supply chain



Dedicated program management team

Why choose nucleotides from TriLink BioTechnologies®

In addition to modified uridine and cytidine triphosphates for the development of mRNA therapeutics, we provide other modified nucleotides and unmodified/standard nucleotides in catalog and custom formats. TriLink's nucleotides offer:

- **Wide selections**—varieties as custom, catalog, modified, and unmodified nucleotides
- **Flexible formats**—customizable aliquots and packaging to suit your application
- **Reliable products**—supported by >25 years of nucleic acid innovation, expertise, and manufacturing
- **Fit for purpose**—manufactured with quality and scales for therapeutic and diagnostic development, from discovery to commercialization

Foundations for successful RNA manufacturing

TriLink offers unmodified ribonucleoside triphosphates (rNTPs) with $\geq 99\%$ purity in both research-use-only (RUO) and good manufacturing practice (GMP) grades, enabling seamless transitions from research and discovery to clinical development. They are manufactured under stringent quality standards in an ISO-9001 certified facility, providing exceptional quality, purity, and consistency.

TriLink's rNTPs are ideally suited for RNA synthesis in research, diagnostics, therapeutics, and vaccine development. These high-quality rNTPs can be used in conjunction with a wide range of modified nucleotides—such as N1-methylpseudouridine and 5-methoxyuridine—and CleanCap co-transcriptional capping technology for robust RNA manufacturing.

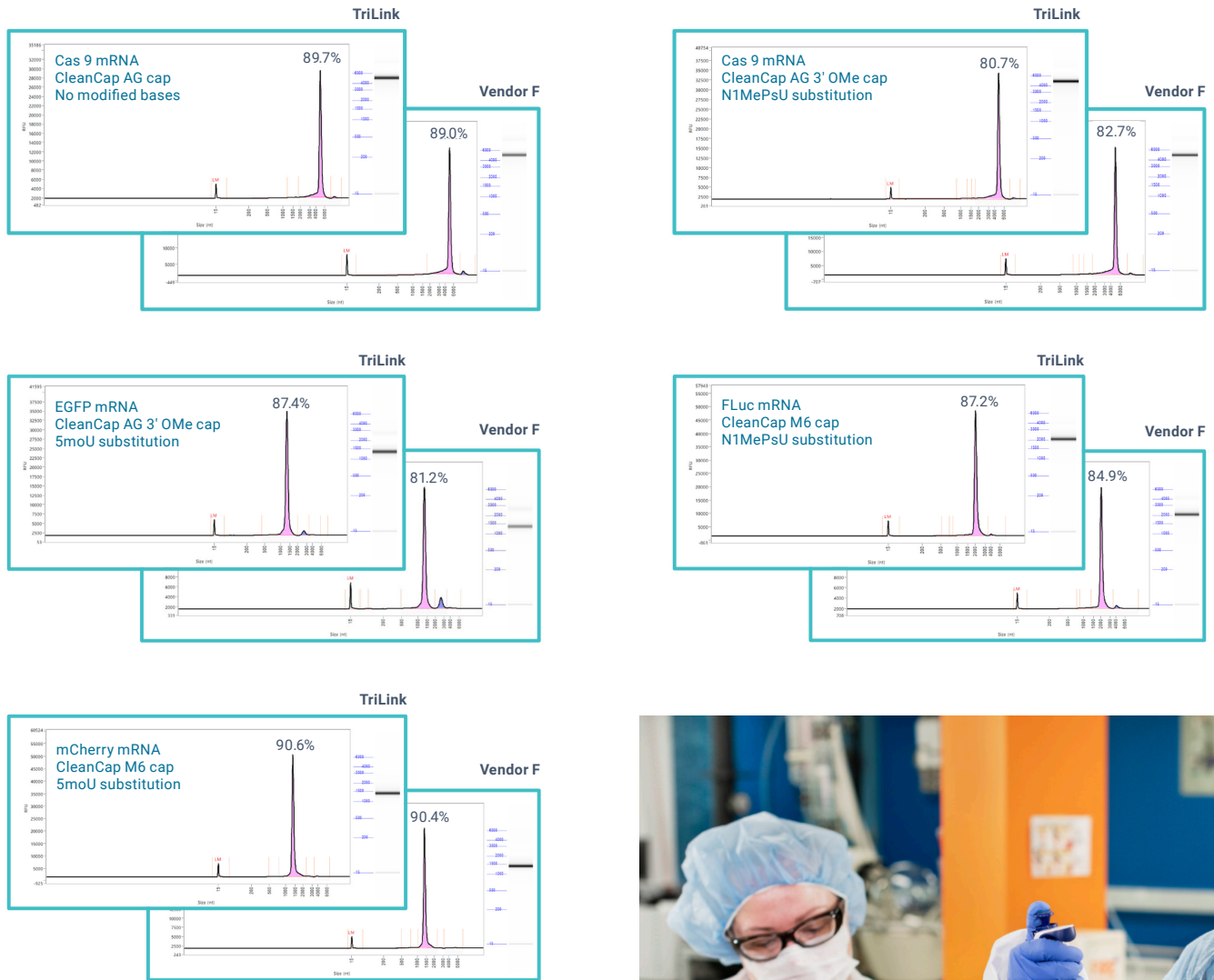


Figure 2. Fragment analyses of five mRNA constructs manufactured using TriLink's or another vendor's unmodified rNTPs. They all exhibit comparable full length's integrity in the range of 80-90% after oligo dT capture. The constructs were capped with CleanCap M6, CleanCap AG 3' OMe, or CleanCap AG analog. In some constructs, uridines were substituted with TriLink's N1-methylpseudouridines (N1MePsU) or 5-methoxyuridines (5moU).



Available modifications of nucleotides to suit your needs

TriLink offers nucleotides with various modifications, whether they are unique to your application, cited in a publication, or well-established in the field. Our offerings on nucleotide modifications range from common nucleobases to sugar modifications and backbone options.

Table 1. Example nucleotide modifications

| Nucleobases | Sugar modifications | Backbone options |
|--|--|---|
| Deoxyribonucleotides | | |
| <ul style="list-style-type: none"> • Adenine • Cytosine • Guanine • Thymine • Uridine • Inosine | <ul style="list-style-type: none"> • 2'-3'-dideoxy • 3'-deoxy | <ul style="list-style-type: none"> • 5'-o-(1-thiotriphosphate) • 5'-o-(1-boranotriphosphate) • 5'-hexylphosphate • 5'-hexaphosphate |
| Ribonucleotides | | |
| <ul style="list-style-type: none"> • Adenine • Cytosine • Guanine • Thymine • Uridine • Pseudouridine • Inosine | <ul style="list-style-type: none"> • 2'-amino • Ara • 2'-azido • 2'-fluoro • 2'-o-methyl • 3'-o-methyl | <ul style="list-style-type: none"> • 5'-o-(1-thiotriphosphate) • 5'-5'-triphosphate |



For specific applications such as epigenetic analyses, DNA damage and mutagenesis assays, photolinking experiments, and dye labeling and attachment, we are here to provide what you need to succeed in your projects. Our capabilities include:

- **Cell proliferation / cancer:** 5-bromo-U, 2-amino-A, 8-chloro-A, 6-aza-U, 2-thio-C, 5-fluoro-U
- **Dyes / labels:** cyanine, dabcy1, biotin
- **Epigenetics / methylation:** 5-formyl-C, 5-carboxyl-C
- **Linker and attachment:** 5-aminoallyl-U
- **Mutagenesis:** 8-oxo-dG, 7-deaza-G
- **Photoreactivity:** 8-azido-A, 2-thio-T, 5-iodo-C

The modifications listed here are not comprehensive. Please contact us at nucleotides@trilinkbiotech.com for your unique interest.

Empowering your innovation with our expertise

Scalable processes and manufacturing

Maintaining scalability allows your project to keep to established timelines, absorb unexpected surges for product, and remain aligned with your clinical research goals. Our 118,000 sq ft facility contains multiple specialized manufacturing suites and support labs, ensuring success across a wide variety of synthesis scales.

Analytical support

With decades of experience, we understand your analytical objectives from process development (PD) to scale-up and GMP manufacturing. TriLink has developed extensive in-house capabilities in method development and analytical testing to ensure a high-quality manufacturing process. We work with you to ensure effective method development to meet your objectives at each stage of product development.

Analytical methods performed on our nucleotides include:

RUO products

- pH
- **Concentration:** UV/Vis
- **Purity:** ^{31}P NMR, AX-HPLC
- **Identity:** ^1H NMR, mass spectrometry

GMP-grade products (in addition to RUO analyses)

- **Characterization:** appearance, residual chemicals
- **Safety:** endotoxin, bioburden
- **Nuclease contamination:** DNase and RNase detection



Ordering information*

| Product | Cat no. | Catalog sizes |
|---|---------|----------------------------|
| Modified uridines and cytidines | | |
| N ¹ -Methylpseudouridine-5'-Triphosphate | N-1081 | 1 µmol, 5 µmol, 10 µmol |
| 5-Methoxyuridine-5'-Triphosphate | N-1093 | 1 µmol, 5 µmol, 10 µmol |
| Pseudouridine-5'-Triphosphate | N-1019 | 1 µmol, 5 µmol, 10 µmol |
| 5-Methylcytidine-5'-Triphosphate | N-1014 | 1 µmol, 5 µmol, 10 µmol |
| rNTP set with N1MePsUTP | N-1506 | 100 µmol |
| rNTP set with 5moUTP | N-1507 | 100 µmol |
| rNTP set with PsUTP | N-1508 | 100 µmol |
| Unmodified nucleotides | | |
| Adenosine-5'-Triphosphate | N-1501 | 10 µmol, 25 µmol, 100 µmol |
| Cytidine-5'-Triphosphate | N-1502 | 10 µmol, 25 µmol, 100 µmol |
| Guanosine-5'-Triphosphate | N-1503 | 10 µmol, 25 µmol, 100 µmol |
| Uridine-5'-Triphosphate | N-1504 | 10 µmol, 25 µmol, 100 µmol |
| Ribonucleoside-5'-Triphosphate Set | N-1505 | 10 µmol, 25 µmol, 100 µmol |
| CleanCap® analogs | | |
| CleanCap® Reagent M6 | N-7453 | 1 µmol, 5 µmol, 10 µmol |

*Additional products, sizes, and formats—including **bulk** and **GMP-grade** options—are available. Please visit trilinkbiotech.com/nucleotides or email nucleotides@trilinkbiotech.com for more information.

Learn more about our nucleotides at trilinkbiotech.com/nucleotides.

Contact us at **800.863.6801** or nucleotides@trilinkbiotech.com to help you identify nucleotides suitable for your application.