CELLSCRIPT™ Posts

Diversity in mRNA Applications

Understanding and Reducing Immunogenicity

High Efficiency mRNA Synthesis

Advances in mRNA Research - mRNA CAR-T

mRNA Research Advancement Awards

FALL 2025

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Welcome to the inaugural issue of CELLSCRIPT™'s news magazine, *CELLSCRIPT™ Posts*. In this magazine you'll find original articles describing trends and breakthroughs in mRNA research and medicine, information about new CELLSCRIPT™ technologies and services, as well as product application notes and links to exciting original research publications by scientists that have incorporated our products into their workflows.

In this issue, we focus on the diversity of mRNA applications through two featured articles, *Understanding and Reducing Immunogenicity in mRNA Downstream Applications* and *Advances in mRNA Research: Breakthrough CAR-T Targeting of Multiple Diseases.* Our Application Note*, High Efficiency mRNA Synthesis and Ultra-Low Immunogenicity Achieved with the T7 mScript™ Complete mRNA Production Systems* provides data demonstrating how our dual workflow approach to mRNA synthesis incorporating modified nucleoside transcription followed by enzymatic Min-Immune™ Gold dsRNA removal can increase translation efficiency ~900% and result in <0.005% (LLOQ) dsRNA in samples. Explore the trending use of plasmid DNA rolling circle amplification as a method for creating DNA templates for RNA transcription in our second Application Note, *TempliAMP™ RCA Kit: Performance analysis against commercial RCA Kits for DNA yield and workflow time.* We're sure you'll find the information presented in this issue exciting and useful.

Finally, we announce a new mRNA research support program, the 2026 CELLSCRIPT™ mRNA Research Advancement Awards, which will provide up to ten (10) Awards, each consisting of $10,000 USD retail value of CELLSCRIPT™ technologies, to enable US researchers to accomplish their mRNA vaccine, cell and gene therapy, mRNA therapeutics or other studies successfully. Application submission deadline is December 15, 2025.

For more information about our products, visit www.cellscript.com. To connect directly with one of our mRNA Experts to discuss your research needs, go to https://www.cellscript.com/contact-an-mrna-expert/.

Happy reading!

Joby Chesnick, PhD MBA

Director of Marketing, CELLSCRIPT™

CELLSCRIPT™

mRNA Research Advancement Awards

Enabling your mRNA breakthrough discoveries

mRNA has reshaped the landscape of healthcare over the last several years through advances in mRNA vaccines, cell and gene therapies, and therapeutics. To support continued advances in the field of mRNA research, CELLSCRIPT™ is soliciting applications for our 2026 mRNA Research Advancement Awards.

What are the CELLSCRIPT™ mRNA Research Advancement Awards?

Up to ten (10) CELLSCRIPT™ mRNA Research Advancement Awards each consisting of $10,000 USD retail value of CELLSCRIPT™ mRNA technologies will be awarded to enable US researchers to accomplish their mRNA vaccine, cell and gene therapy, mRNA therapeutics or other studies. Awards are not transferable to another person or institution.

- $10,000 USD retail value of CELLSCRIPT™ mRNA Products
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- Scientists in the USA who are conducting mRNA research at a university, biotechnology or pharmaceutical company, or government or private research institution.
- Researchers must hold a BS, MS or PhD in the life sciences or related field.
- Researchers must hold a permanent full-time position at their institution.
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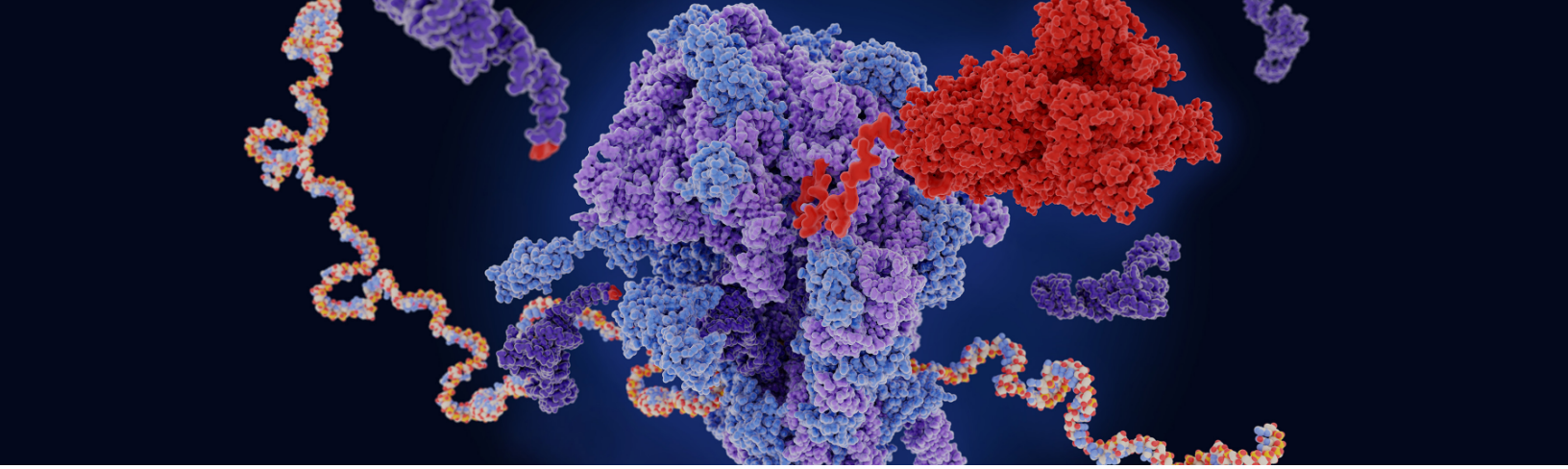

Article

Understanding and Reducing Immunogenicity in mRNA Downstream Applications

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The use of mRNA as a therapeutic agent originated in 1990 when Wolff et al. successfully expressed proteins in target organs by directly injecting mRNA.[1] Subsequently, further advances in molecular biology resulted using *in vitro*-produced mRNA for gene therapy. Currently, mRNA as biomedicine is being explored as vaccines for infectious diseases and cancers, and other therapeutics such as in regenerative medicine and genome editing.[1] mRNA can translate into proteins that induce an innate immune response, which is beneficial for vaccination and immunotherapy applications. However, in non-immunotherapy applications, the intrinsic immunostimulatory activity of mRNA is undesirable since it can reduce target protein expression.

mRNA for Therapeutics vs mRNA for Vaccine Development

The development of mRNA therapeutics is different from the development of mRNA vaccines. For mRNA vaccines, only a minimal amount of protein production is needed, as the immune system can amplify the antigenic signal through cell-mediated and antibody-mediated immunity. On the other hand, mRNA therapeutics may require as much as a 1,000-fold-higher level of protein to reach a therapeutic threshold to engage a particular target pathway, cell, tissue or organ.[2] Furthermore, repeated dosing, which is often required in the treatment of chronic diseases, activates innate immunity, which can reduce therapeutic protein expression.

Activation of the Innate Immune System

When a eukaryotic cell detects foreign RNA, it responds to it as it would a viral infection. The innate immune system has RNA sensors which detect foreign RNA. Several RNA-binding proteins in mammalian cells control immune reactions by modulating gene expression, splicing, nuclear export, mRNA modification, translation, and degradation.[1]

Molecular mechanisms that distinguish self and non-self RNAs initiate an antiviral response, which includes the expression of type I interferon (IFN) and IFN-stimulated genes.[1] Pathogen-associated molecular patterns (PAMPs) are the main RNA response mechanism via recognition by pattern recognition receptors (PRRs).[1] PRRs include toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated protein 5 (MDA5), cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) second messenger system, and NOD-like receptors (NLRs).[1] Activation of RIG-I and MDA5 causes oligomerization on double-stranded RNA (dsRNA), with ubiquitin, which is an important part of this process. Oligomerized and ubiquitinated RIG-I or MDA5 with dsRNA interact through CARD domains with mitochondrial antiviral signaling (MAVS) proteins that then activate transcription factors NF-κB and IFN-regulatory factors IRF3/7.[1]

The standard method by which mRNA enters cells is by endocytosis, where it binds receptors in endosomes for RNA recognition such as TLR3 for dsRNA and TLR7/8 for single-stranded RNA (ssRNA).[3] Additionally, other receptors in the cytoplasm including RIG-I, MDA5, NLRP3, and NOD2, recognize exogenic RNA.[3] Subsequently, transcription factors IRF3 and NF-κB are activated. NF-κB plays an important role in launching the innate immune response mechanism that targets viral nucleic acids.[3] NF-κB activates transcription of proinflamma tory genes that leads to the synthesis of cytokines, chemokines, cell cycle regulators, anti-apoptotic factors, and adhesion molecules.[1] IRF3 activates the transcription of IFN genes that inhibit the translation of exogenous RNA via interferon synthesis.[3] NF-κB triggers the expression of IL-1β and IL-6, whereas IRF3 triggers the expression of IFNβ and INFγ, and IRF7 initiates the

expression of IFNα.[4] IFNs are secreted out of the cell and bind to their receptors, resulting in a positive feedback signaling loop that increases local secretion of IFNs and other immunostimulatory factors, leading to the antiviral response, RNA degradation, and suppressed protein translation.[5]

One of the key considerations for mRNA therapeutics, particularly the development of mRNA vaccines, is the activation of RIG-I and MDA5 because their main ligand, dsRNA, is a byproduct of IVT. dsRNAs activate RIG-I and MDA5 in different ways: RIG-I preferentially recognizes short sequences, and MDA5 recognizes long dsRNA sequences.[6]

NOD-Like Receptors and Inflammation

Another equally important group of RNA pattern recognition receptors is NOD-like receptors (NLRs). NLRs are categorized into groups according to their ability to form inflammasomes.[1]

Inflammation is an important physiological response. Any challenge to the body's homeostasis may trigger inflammation at the local or systemic levels. The innate immune system's role is to generate a protective response against signals of homeostasis disruption.[7] The term "inflammasomes" emerged from the research of Martinon et al. in 2002, that described the assembly of supramolecular structures in the cytoplasm of activated immune cells driving subsequent systemic immune responses and inflammation.[8] Inflammasomes are cytoplasmic multiprotein complexes that can be activated by numerous endogenous and exogenous stimuli. Appropriate inflammasome activation is critical for a host organism to cope with foreign pathogens or tissue damage, however, this activation can also cause uncontrolled tissue responses that may contribute to various diseases, including autoinflammatory disorders, cardiometabolic diseases, cancer, and neurodegenerative diseases.[9]

NOD2 controls cytokine expression through its activation of NF-κB and also takes part in the response to exogenous dsRNA and ssRNA.[9] NLRP3 activation has a detrimental effect on mRNA transfection efficiency, and its activation may contribute to some of the adverse reactions to mRNA biomedicines such as the mRNA-1273 COVID-19 vaccine.[10,11]

Immune Response via the GMP-AMP synthase STING pathway

The cyclic GMP–AMP synthase (cGAS)/stimulator of IFN genes (STING) pathway may be important in the cellular immune response through its role of innate sensing of cytosolic RNA.[12] STING interacts with RIG-I and MAVS, which are key components of the RNA sensing pathway and may participate in the RNA virus-induced cytokine production.[13,14] RIG-I and STING have been reported to participate in the RNA virus-induced defence responses.[13,15] STING initiates global translation inhibition to restrict the production of both viral and host proteins in a RIG-I/MDA5-dependent but MAVS-independent manner.[16] Thus, the recognition of an RNA virus infection by RIG-I/MDA5 probably results in two distinct responses—one is mediated by MAVS to induce IFNs and cytokines, and the other is mediated by STING to inhibit translation. Thus, the activation of cGAS/STING pathway may lower the efficiency of mRNA translation and mediate cytotoxicity.

mRNA Design Strategies for Reducing Immunogenicity

The activation of RIG-I and MDA5 in non-immunotherapeutic approaches can be reduced with standard methods for decreasing the immunogenicity of RNA, by 5′ capping, reducing the uridine (U) content via nucleoside modifications, 3′-poly(A) tailing, reducing the number of double-stranded products of *in vitro* transcription, and inhibiting PRRs.[17]

Designing mRNA with optimized untranslated regions (UTRs) and codon usage can enhance translation efficiency and reduce immunogenicity. Synthetic mRNA consists of a single-stranded molecule from 5′ to 3′ that includes: a 5′-cap structure, a 5′ untranslated region (UTR), a coding Open Reading Frame (ORF) sequence, a 3′ UTR, and a 3′-poly(A) tail.[18] The ORF sequence plays a critical role in immunogenicity. The coding sequence for the target protein needs to be optimized to protect the mRNA from exonuclease degradation, such as through strategically increasing guanine and cytosine (GC) content, reducing uracil (U), and removal of rare codons.[19] In addition, long 3′ UTRs have been associated with increased dsRNA formation and subsequent inflammation via activation of dsRNA-sensing PRRs such as MDA5, PKR, and TLR3.[20]

Another main method to reduce immunogenicity is the use of modified bases such as pseudouridine or N1-methyl-pseudouridine (N1meΨ), which changes mRNA secondary structures (hairpins) thus preventing TLR3 or RIG-I from recognizing the mRNA, which in turn causes the down-regulation of type I IFNs.[21] The inclusion of N1meΨ has been shown to decrease dsRNA immunogenicity.[18] In some studies, this approach has been shown to be better than chromatographic purification for the removal of dsRNA from the original product, thereby reducing immunogenicity.[21, 22]

In addition, removing dsRNA from a mRNA mixture using high-performance liquid chromatography improves translation of mRNA up to 1,000-fold and that purified mRNA causes less induction of IFNs and inflammatory cytokines.[21] Whether or not nucleotide base modifications are necessary in mRNA to reduce immunogenicity in addition to dsRNA removal is an ongoing discussion. dsRNA, compared to other exogenous RNAs, is the main trigger of immunogenic cellular response.[1]

A study by Mulroney et al. (2024), showed that despite the benefits of modified nucleosides on immunogenicity and protein translation, the incorporation of N1-methyl-pseudouridine in mRNA induces a +1 ribosomal frameshift both *in vitro* and *in vivo*. This mistranslation could lead to the presentation of +1 frameshifted antigens to T cells, potentially triggering off-target cellular immune responses.[23]

Furthermore, adding a poly(A) tail may lower mRNA immunogenicity by lowering U content and shielding mRNA in the

sequence through circularization.[24] The poly(A) tail can bind to polyadenosyl-binding proteins (PABP), which interacts with the N-terminal region of EIF4G, forming a complex that interacts with the 5′ cap and EIF4E complex, resulting in an circularized mRNA structure which protects the mRNA from nucleolytic degradation and enhances translation efficiency by ribosome reutilization.[25]

Furthermore, circular mRNA (circmRNA), a covalent closed loop formed by reverse splicing of the 3′ end to the 5′ end of mRNA, as an alternative to traditional linear mRNA, is being considered as the next-generation of mRNA therapeutics due to its stability and low immunogenicity.[26,27]

Presently, a dual strategy is recommended to reduce immunogenicity of RNA, i.e., the use of modified nucleotides and dsRNA removal. While modified nucleotides primarily reduce the mRNA's inherent immunostimulatory properties, dsRNA removal ensures that the final product is free from a major source of immune activation. Nelson, J. et al. (2020), showed that dsRNA removal alone is not sufficient at avoiding immune activation and that it is the combination of uridine modification (N1meΨ for uridine) and dsRNA removal that are required to generate a furtive mRNA-based drug product.[28] This is particularly important for therapies that require repeated administrations, as immune responses have memory, which could render subsequent administrations less effective over time.[28]

Conclusion

In conclusion, a multitude of cellular immune response pathways are triggered in the presence of foreign RNA. mRNA biomedicines, while promising, can trigger an innate immune response. The innate immune system recognizes mRNA as foreign and this recognition initiates a cascade of events, including the production of interferons and inflammatory cytokines. While this response is crucial for vaccine efficacy, it can also lead to adverse effects and limit the therapeutic potential of mRNA for repeated dosing. Researchers are actively investigating the complex relationship between mRNA and the innate immune system to optimize mRNA-based therapies. However, further research is still needed to refine mRNA technology and unlock its full potential for treating a wide range of diseases. As the field of RNA-based therapeutics advances, a deeper understanding of the key factors in the immune system and how they can be mitigated is critical for developing safe and effective and drugs.

CELLSCRIPT™ Products for Immunogenicity Reduction

CELLSCRIPT™ sets a new standard in IVT mRNA production through a dual optimization strategy that combines:

- **INCOGNITO™ mRNA Kits** – mRNA transcription using chemically modified nucleotides minimizes innate immune recognition and enhances mRNA stability and translation.
- **Min-Immune™ Gold dsRNA Removal Kit** – Next-generation enzymatic purification reduces residual dsRNA to <0.005% (LLOQ), significantly lowering immunogenic risk.

Together, these technologies enable the production of high-quality, low-immunogenicity mRNA optimized for therapeutic and translational applications. ■

Learn more: CELLSCRIPT™ Immunogenicity Reduction

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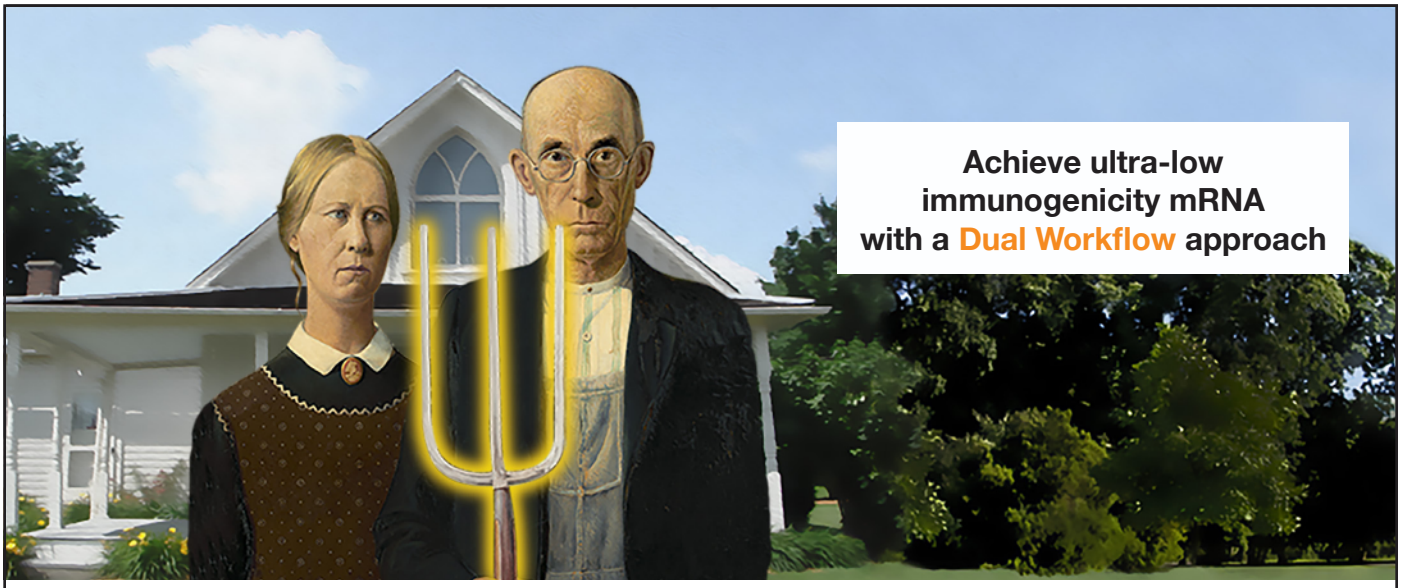

Synthesized mRNA with ultra-low immunogenicity.

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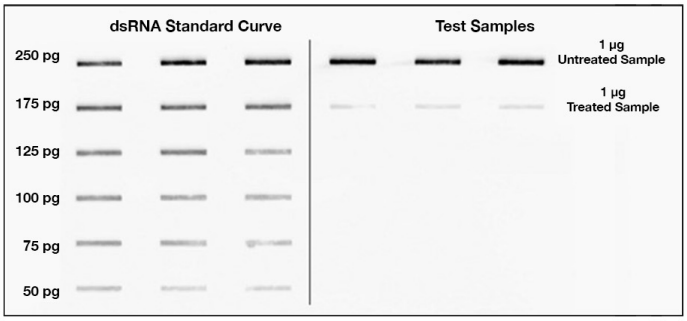
Step 1: INCOGNITO™ T7 mScript™ mRNA Synthesis

- High-yield mRNA production with modified nucleotides (Ψ or N1meΨ) for immunogenicity reduction
- 100% post-transcriptional capping efficiency and tail lengths of even ~300+ bases are possible for maximum stability
- Scalable from research to production volumes

Step 2: Min-Immune™ Gold dsRNA Removal

- High efficiency post-transcriptional dsRNA removal to <0.005% (LLOQ) of sample (Figure 1)
- No loss of precious ssRNA yield
- Reduces immunogenicity to ultra-low levels for critical applications

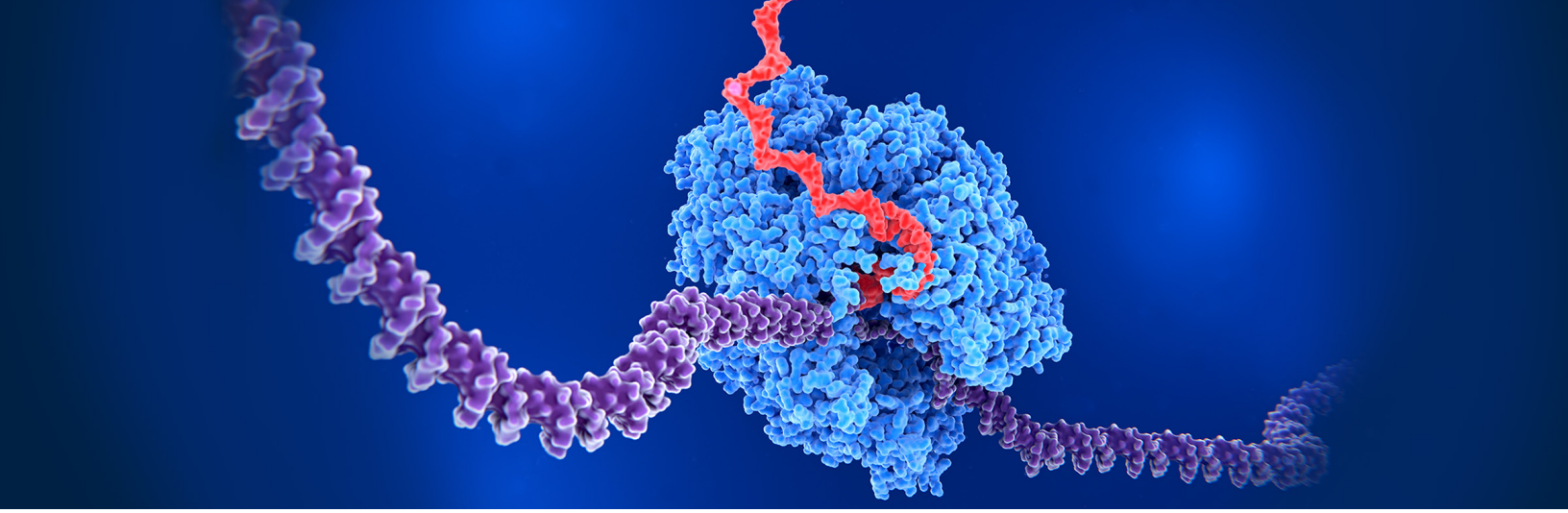


Sample	% dsRNA Content
Untreated RNA	0.033%
Min-Immune™ Gold dsRNA Removal Kit Treated Sample	<0.005% (LLOQ)

Figure 1. Comparison of Min-Immune™ Gold-treated 1.4 kb pseudouridine (Ψ)-containing RNA sample to an untreated sample (right panel) and dsRNA standards (left panel). Min-Immune™ Gold-treated dsRNA is reduced below the limit of quantitation (<0.005%) after treament.

Learn More

www.cellscript.com/immunogenicity-reduction



Application Note

High Efficiency mRNA Synthesis and Ultra-Low Immunogenicity Achieved with the T7 mScript™ Complete mRNA Production Systems

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Introduction

Messenger RNA (mRNA)-based therapeutics and vaccines have emerged as a transformative platform in molecular medicine, offering rapid, scalable, and cell-free production of biologically active proteins.[1] The success of mRNA vaccines during the COVID-19 pandemic has accelerated interest in optimizing mRNA synthesis, modification, and purification to enhance translational efficiency and minimize innate immune activation. *In vitro* transcription (IVT) using bacteriophage T7 RNA polymerase is a widely adopted method for producing synthetic mRNA. However, IVT reactions often generate double-stranded RNA (dsRNA) byproducts, which can trigger potent antiviral responses through pattern recognition receptors such as RIG-I and MDA5.[2-4] These immune responses can reduce protein expression and compromise the safety and efficacy of mRNA-based therapeutics. To address this, chemical modifications such as N1-methyl -pseudouridine (N1meΨ) substitution for uridine, and post-transcriptional purification strategies to remove dsRNA, have been employed to reduce immunogenicity and improve mRNA stability and translation.[5-9]

In this study, we synthesized firefly luciferase mRNA using the T7 mScript™ Complete mRNA Production Systems, incorporating either canonical UTP or modified N1meΨTP. We evaluated the impact of dsRNA removal using the included Min-Immune™ Gold dsRNA Removal Kit module on mRNA quality and expression potential. Quality control assays were performed to assess capping efficiency, poly(A) tail length, and overall mRNA integrity. This work contributes to the growing body of knowledge aimed at refining mRNA production workflows for research and therapeutic applications and positions the T7 mScript™ Complete mRNA Production Systems as highly suitable for synthesis of high-quality mRNA devoid of dsRNA and amenable for use in downstream applications such as mRNA vaccine development and cell and gene therapy research.

The Challenge

Unmodified mRNA can result in mRNA that has lower stability, activates the immune system, and reduced translational efficiency. Additionally, immune responses caused by dsRNA byproducts generated during *in vitro* transcription can compromise the safety and efficacy of mRNA-based therapeutics which is a major challenge for downstream mRNA therapeutic applications.

The Solution

We present an optimized dual strategy to create high-yield single-stranded mRNA using mRNA modified with N1-methyl-pseudouridine (N1meΨ) that produces markedly higher protein expression using the INCOGNITO™ T7 mScript™ Complete N1meΨ-mRNA Production System compared to unmodified transcripts. The removal of double-stranded RNA contaminants using the Min-Immune™ Gold dsRNA Removal Kit procedure further improves protein expression,

and significantly reduces immunogenicity. This integrated dual workflow seamlessly combines high-efficiency mRNA transcription with N1meΨ modification and dsRNA removal, providing an optimal solution for generating ultra-low immunogenicity mRNA for downstream applications.

Materials and Methods

In vitro Transcription of Firefly Luciferase mRNA

Firefly luciferase mRNA was synthesized using the T7 mScript™ Complete Standard mRNA Production System using canonical nucleotides (Cat. No. MSCC250225) and the INCOGNITO™ T7 mScript™ Complete N1meΨ-mRNA Production System using N1-methyl-pseudouridine-5′-triphosphate [N1meΨTP] (Cat. No. IMCMY250225) following the manufacturer's protocol. Each reaction was carried out at 37°C for 30 minutes using a linearized DNA template encoding the firefly luciferase gene.

Following transcription, the IVT RNA was purified by ammonium acetate (NH_4OAc) precipitation, washed with 70% ethanol, resuspended in RNase-free water, and quantified by spectrophotometry to confirm yield and purity. Post-transcriptional modifications were performed sequentially according to the kit instructions. 5′-end capping reactions were performed to generate a Cap 1 structure, followed by 3′-end polyadenylation. The final mRNA product, approximately 1.8 kilobases in length, was purified again and quantified by spectrophotometry to confirm yield and purity.

Double-Stranded RNA (dsRNA) Removal

To assess the impact of dsRNA on mRNA expression, an aliquot of untreated mRNA was reserved as a control for presence and quantity of contaminating dsRNA which is produced during the transcription reaction. Double-stranded RNA is a molecule composed of two complementary strands of RNA, held together by hydrogen bonds. It is a common contaminant produced during IVT. This dsRNA is unwanted because it triggers innate immune responses and reduces the efficiency of protein translation. The remaining mRNA was subjected to dsRNA removal using the kits according to the kit instructions. Briefly, RNA was incubated with the kit reagents at 37°C for 1 hour. Following treatment, RNA was purified by NH_4OAc precipitation, washed with 70% ethanol, resuspended in RNase-free water, and quantified by spectrophotometry.

mRNA Quality Assessment

The integrity and quality of the synthesized mRNA were evaluated using CELLSCRIPT™'s EZ-QC™ mRNA quality control assay kits (Table 1). These included the EZ-QC™ mRNA Cap 1 Efficiency Assay (Cat. No. ONE240910), the EZ-QC™ mRNA Capping Efficiency Assay Kit (Cat. No. ACE240910), and the EZ-QC™ mRNA Poly(A) Tail Length Assay Kit (Cat. No. PAT240910). All assays were performed in accordance with the manufacturer's protocols.

Table 1. mRNA Quality Assessment with EZ-QC™ Kits.

		Assay Results	
mRNA Quality Assay	**Catalog #**	**UTP**	**N1meΨTP**
EZ-QC™ mRNA Capping Efficiency Assay	ACE240910	98% Capped	95% Capped
EZ-QC™ mRNA Cap 1 Efficiency Assay	ONE240910	94% Cap 1	93% Cap 1
EZ-QC™ mRNA Poly(A) Tail Length Assay	PAT240910	148-A's	166-A's

Luciferase Transfection and Assay Methods

The firefly luciferase mRNAs were transfected in duplicate into human THP-1 cells (ATCC), a monocytic cell line. The cells were expanded in serum-containing media and plated 24 hours prior to transfection. $2x10^5$ cells were plated per well and transfected in duplicate with 1.5 µg of each mRNA and 2.5 µl Lipofectamine™ MessengerMax™ Transfection Reagent (Thermo Fisher Scientific) in 100 µl of Opti-MEM™ medium (Thermo Fisher Scientific). After a 24-hour incubation at 37°C with 5% CO_2, the cells were collected, washed, and lysed with Reporter Lysis Buffer (Promega). The lysates were assayed with an equal volume of ONE-Glo™ EX Luciferase Assay Substrate (Promega). Average relative light units (RLUs) from duplicate assays on duplicate samples were plotted.

RT-qPCR to detect Innate Immune Response Methods

Human THP-1 cells were transfected in duplicate as described. After a 24-hour incubation, the cells were collected, washed and total cellular RNA was isolated from each well using the MasterPure™ Complete DNA and RNA Purification Kit (Biosearch Technologies). Equivalent amounts of RNA were amplified by RT-qPCR to detect differences in the cellular response to mRNA transfection. The purified cellular RNA was reverse transcribed and amplified using the iTaq Universal SYBR® Green One-Step Kit (Bio-Rad) with 9 primer sets using the Bio-Rad CFX 96 real-time cycler. The results from the test samples were averaged, normalized to GAPDH expression, and presented relative to the results from control cells transfected without any mRNA. The relative normalized expression or ΔΔCq was plotted for each primer set with the SEM.

Results

Luciferase Assay to Determine Protein Expression with N1meΨ-Modified mRNA

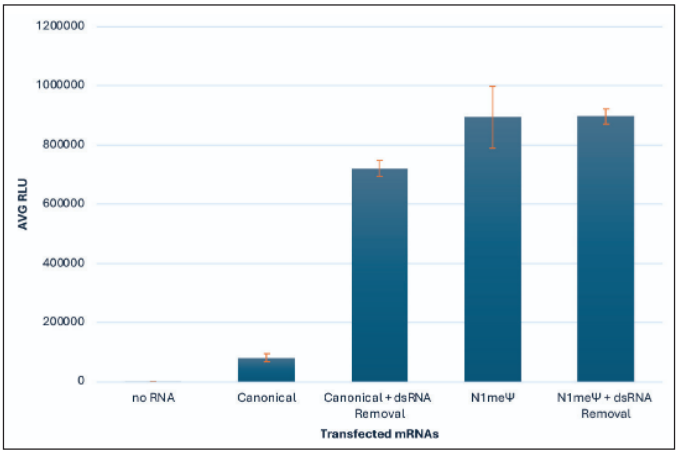

Figure 1. Measurement of luciferase activity in THP-1 cells 26 hours post-transfection. The combination of N1meΨ modification and dsRNA removal resulted in the highest luciferase expression.

N1meΨ-modified mRNA yielded the highest luciferase expression, with an average of ~895,000 RLU (Figure 1). Notably, dsRNA removal using the Min-Immune™ Gold module significantly enhanced expression of canonical unmodified mRNA (~721,000 RLU), approaching levels observed with N1meΨ-modified transcripts. Comparing canonical mRNA to canonical mRNA +dsRNA removal, there was a 8.87 fold increase or a 887% increase in luciferase expression. The combination of N1meΨ modification and dsRNA removal resulted in the highest expression (~898,000 RLU), suggesting additive benefits of chemical modification and purification. Min-Immune™ Gold purified luciferase mRNA samples demonstrated significantly reduced amounts of dsRNA vs controls, with implications for highly improved translation of mRNA produced with canonical nucleotides.

RT-qPCR to Detect Innate Immune Response

Primer pairs were designed to RNA-responsive immune sensor genes including toll like receptors (TLR3, TLR7), dsRNA receptors (RIG-I, MDA5), interferon beta (IFNB1), nucleic acid receptors stimulated by the interferon response (OAS1, PKR) and a proinflammatory cytokine (TNF) (Figure 2). These and other genes, when elevated, indicate transfected RNA has been detected by the cell. The stronger the response or expression of these genes, the larger the cascades of type one interferons, protein translation inhibition, RNA degradation and cell death.

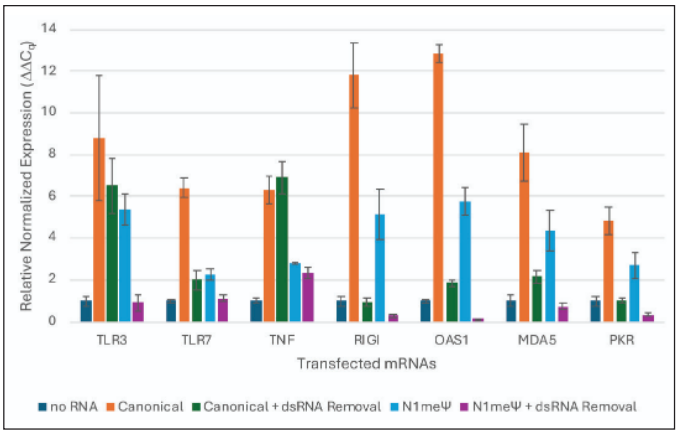

Figure 2. RT-qPCR measurement of relative normalized expression of RNA-responsive immune sensor genes. Canonical unmodified mRNA had the highest expression of immune sensors, while N1meΨ modified mRNA and dsRNA mRNA had the lowest.

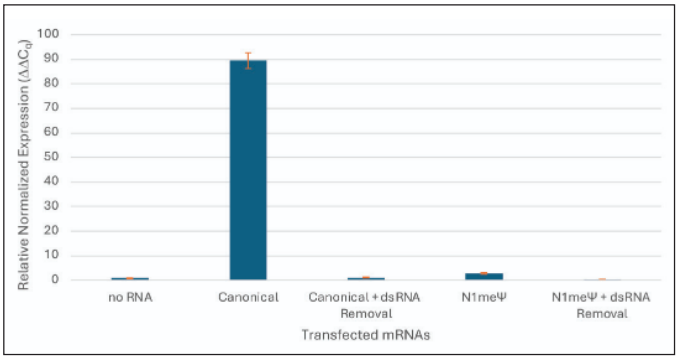

Figure 3. RT-qPCR relative normalized expression of innate immune response genes. Canonical mRNA had the highest immune response while N1meΨ mRNA in combination with dsRNA removal had the lowest innate immune response.

N1meΨ mRNA that has been purified with the Min-Immune™ Gold module of the kit results in almost no detectable innate immune response (Figure 3).

Conclusion

The results of this study demonstrate that both uridine analogs and post-transcriptional purification significantly influence the translational efficiency and immunogenicity of synthetic mRNA in human immune cells. Using firefly luciferase as a reporter, and the INCOGNITO™ T7 mScript™ Complete N1meΨ-mRNA Production System, we observed that mRNA modified with N1meΨ produced markedly higher protein expression compared to unmodified transcripts. This enhancement is consistent with previous reports showing that N1meΨ incorporation reduces innate immune recognition and improves ribosomal engagement, thereby increasing translation efficiency.

Importantly, the removal of dsRNA contaminants using the Min-Immune™ Gold purification procedure significantly improved luciferase expression by 887%, particularly for unmodified mRNA. This suggests that dsRNA byproducts generated during *in vitro* transcription are a major source of translational inhibition, likely through activation of innate immune sensors such as RIG-I and MDA5. The additive effect observed when combining N1meΨ modification with dsRNA removal underscores the value of a dual strategy to optimize mRNA performance.

RT-qPCR analysis revealed that unmodified mRNA strongly activated innate immune genes, including TLR3, RIG-I, OAS1 and IFNB1 indicating a robust immune response. In contrast, mRNA modified with N1meΨ triggered a much weaker immune response. When N1meΨ-modified mRNA was also purified to remove dsRNA, immune activation was nearly eliminated. These results show that unmodified or impure mRNA can stimulate immune pathways, but chemical modification and purification are effective strategies to reduce this response.

Together, these results support the use of the INCOGNITO™ T7 mScript™ Complete N1meΨ-mRNA Production System that incorporates N1meΨ and rigorous purification protocols free of dsRNA contamination to enhance the safety and efficacy of synthetic mRNA. This has important implications for the development of mRNA-based therapeutics and vaccines, particularly in contexts where immune activation must be minimized, such as protein replacement therapies or gene editing applications. ■

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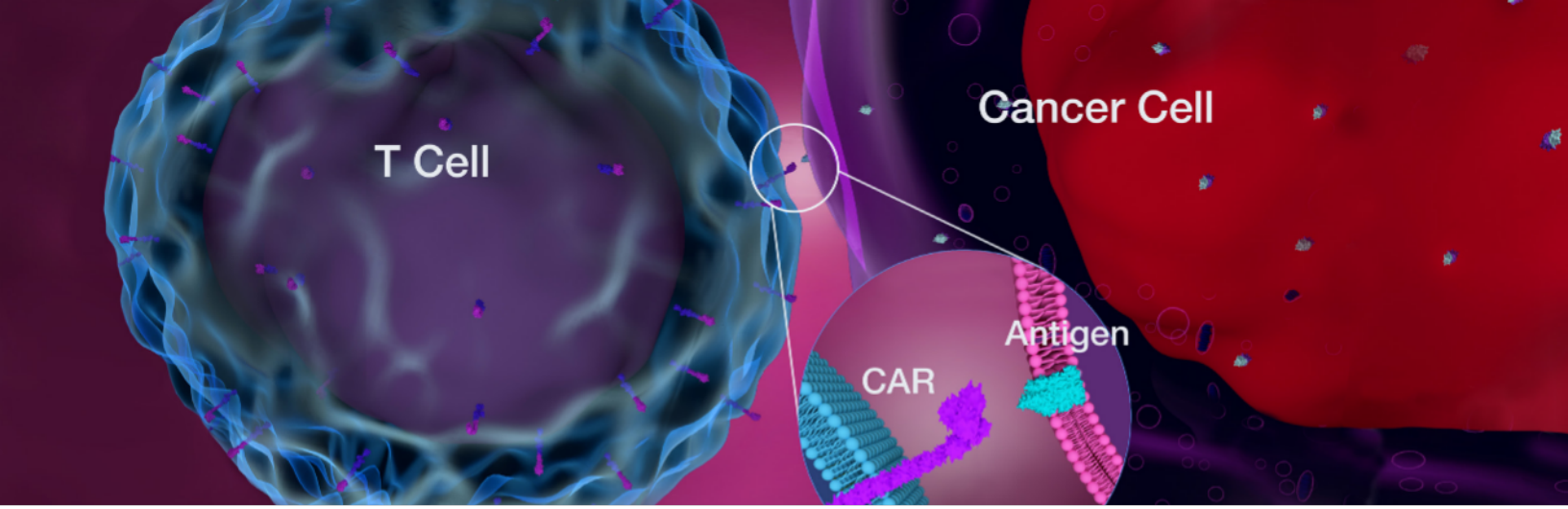
Test Your Knowledge

Submit your contact information and answers to be entered* to win a $25 Amazon gift card

*All answers must be correct for contest eligibility

1. How many mRNAs are contained in the INCOGNITO™ iPSCMax™ Ψ-mRNA Reprogramming Mix?
2. The Adenylator™ DNA/RNA 5′-Adenylation Kit produces how much more output than competitor kits?
3. What is the trademarked name of CELLSCRIPT™'s line of modified nucleoside transcription kits?
4. Which DNA polymerase powers the TempliAMP™ RCA Kit?
5. The EZ-QC™ mRNA Assay Kits use what standard laboratory technique for assay readout?

Submit Your Answers



Article

Advances in mRNA Research: Breakthrough CAR-T Targeting of Multiple Diseases

Fred Hyde, PhD, CELLSCRIPT™, 726 Post Road, Madison, WI 53713 USA

Novel Use of mRNA Therapeutics

mRNA was first discovered decades ago as a molecule that is transcribed from DNA and translated into protein.[1] *In vitro* transcription (IVT) of eukaryotic RNA has five main components: a 7-methyl guanosine (m^7G) 5′-cap structure, a 5′ untranslated region (UTR), an open reading frame that encodes a protein of interest, a 3′ UTR, and a 3′-poly(A) tail.[2] RNA polymerases participate in the transcription of RNA using linear DNA templates (e.g., PCR products and restricted plasmids) containing a specific promoter.[1] For synthetic mRNA production, the cap and poly(A) tail can be added to RNA co-transcriptionally or post-transcriptionally via enzymatic treatments.

Recent advances in addressing stability, translational efficacy, and immunogenicity of the mRNA has opened the possibilities of using mRNA for a broad range of potential therapeutic applications.[3] To reduce immunogenicity and increase translation efficiency, naturally occurring modified nucleotides (e.g., pseudouridine, N1-methyl-pseudouridine, 2-thiouridine, 5-methyluridine, 5-methylcytidine, or N6-methyladenosine) can be incorporated into mRNA sequences during the IVT reaction.[3] In addition, removal of double-stranded RNA (dsRNA) byproducts can further reduce immunogenicity.[3] Other engineering strategies include optimization of UTR and codon sequences, cap types, and the length of poly(A) tails.[3]

Chimeric Antigen Receptor Technology

Immunotherapy has become an accepted way to treat many different advanced or previously untreatable cancers, particularly blood cancers.[4] The concept of a chimeric T-cell receptor (TCR), which combines antibody-derived variable regions (VH/VL) with TCR derived constant regions, was first reported in 1987 by immunologist Dr. Yoshikazu Kurosawa and his team at the Institute for Comprehensive Medical Science.[5] Two years later, in 1989, immunologist Dr. Zelig Eshhar and his colleagues at the Weizmann Institute of Science described a similar approach to redirect T-cells to recognize antigens in a non-major histocompatibility complex (MHC)-restricted manner.[5]

CAR-T cell therapy is a unique form of immunotherapy in that it is made from T-cells, which are the body's primary mechanism for killing infected or diseased cells.[4]

T-cells are collected and sent to labs for genetic engineering changes that allow them to produce special surface proteins called chimeric antigen receptors (CARs). CARs enable the T-cells to attach to specific antigen proteins present on any cell that presents that antigen, including cancerous or diseased cells (Figure 1). This process significantly improves T-cells' ability to kill diseased cells. While CAR-T cell therapy has been used primarily in oncology, it is not limited to cancer treatment and is being explored for the treatment of other diseases such as autoimmune diseases, fibrotic diseases, and infectious diseases.

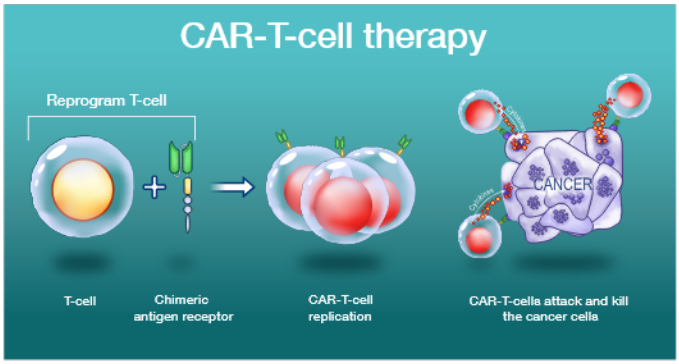


Figure 1. The T-cell to CAR-T cell transformation in cancer therapy.

Convergence of CAR-T and mRNA Therapeutics

mRNA can be rapidly synthesized *in vitro* and used along with cellular machinery in the cytoplasm to theoretically make any protein without transgene integration.[6] With the recent optimization of mRNA synthesis techniques to reduce immunogenicity, enhance stability, and improve protein translation, these strategies have been used to deliver CAR mRNA to T-cells, contributing to mRNA-based CAR therapy.[1] What is crucial in this type of therapy is that special attention must be given to mRNA purity with the removal of dsRNA contaminants for maximum effectiveness.[7]

The workflow for a standard CAR-T oncology therapy workflow consists of:

1. **Isolation and preparation of blood stem cells for mRNA transfection.** This workflow consists of preparing cells to receive mRNA encoding the chimeric antigen receptor gene as an mRNA.
2. **Preparation of plasmid DNA sequence as the mRNA template** encoding the chimeric antigen protein to be targeted by the chimeric antigen receptor.
3. **Synthesis and purification of mRNA created from the antigen gene of interest.** CELLSCRIPT™'s offerings for this step include (1) T7 mScript™ Complete Standard mRNA Production System (with Min-Immune™ Gold dsRNA Removal System included for ultra-low immunogenicity) or (2) the T7 mScript™ Standard mRNA Production System V2.
4. **Transfection of the prepared stem cells with the mRNA,** to express the anti-tumor protein on the cell surface.
5. **Selection and growth of the CAR-containing cells *in vitro,*** harvesting the novel CAR-containing cells and testing of binding capacity of the CAR-T cells to the tumor cells using standard immunological *in vitro* assay techniques.
6. **After screening, selection of the most potent CAR-T cells**, exposure of the tissues/tumor cells to the CAR cells by IV injection into the patient either systemically or by targeted site injection to allow binding of the differentiated T-cells to the antigen-containing tumor cells in the patient.
7. **Monitoring of treatment** to decrease/removal of tumor cells to follow efficacy of treatment.

While T-cell engineering with CAR mRNA is relatively rapid and low-cost to manufacture compared to other therapies, it requires a higher mRNA dosage and repeated dosing for a persistent therapeutic effect.[1] Technologies are being developed to address these bottlenecks through the use of sustained-release mRNA delivery systems, mRNA sequence optimization, self- and trans-amplifying RNA, and circular RNA to increase CAR expression time.[1] In addition, the use of artificial intelligence, machine learning, and modelling of mRNA construct designs and their delivery systems is enhancing the development of mRNA-based CAR therapy.[1]

A Deeper Understanding of CAR-T

Many cancer patients relapse after CAR-T therapy because of a lack of CAR-T persistence.[8] In a 2024 research article published in *Nature* entitled, "Fate induction in CD8 CAR T cells through asymmetric cell division", Lee, C.S. et al., explored the potential of long-term CAR-T persistence, long-lived CAR-T memory and immunosurveillance, and its role in improving therapeutic outcomes through cancer recurrence prevention.

In the study, the research team explored human CD8 CAR-T cells, and the asymmetric cell division (ACD) of T-cells that results in different fates for their daughter cells after target antigen encounter. As a result of ACD, distinct subtypes developed for resulting daughter cells depending upon whether they were positioned proximally or distally to the antigen presenting T-cell. The daughter T-cells proximal to the antigen presenting cell were more likely to differentiate into a short-lived effector T (Teff) cells, whereas the distal-daughter cells became long-lived memory cells with a distinct molecular profile.[8] They found that the global surface proteome underwent asymmetric sorting during the first cell division and identified ACD as a mechanism for the generation of human memory CAR-Ts along with transcriptional, surface proteomic and metabolic profiles of memory and effector precursors.[8] Their data mechanistically linked differential fate trajectories to both cell-intrinsic transcriptional regulation after ACD and asymmetric partitioning of RNA during ACD. The observations and results of this study serve as a framework for optimization of CAR-T and other T-cell immunotherapies.

mRNA Technology for CAR-T Research

For their research, Lee, C.S. et al., transcribed RNA from linear double-stranded DNA templates *in vitro* using CELLSCRIPT™'s T7 mScript™ Standard mRNA Production System. This System enables high-yield canonical mRNA transcription incorporating post-transcriptional capping with ~100% efficiency and poly(A) tail synthesis of variable lengths from 50-300+ A's.

Post-transfection, capped and tailed mRNA has increased stability and translation efficiency in most eukaryotic cell lines. The mScript™ System improves upon co-transcriptional capping and tailing methods by ensuring virtually 100% transcript capping and enabling users to define their desired tail lengths, even greater than 300 A's. In this way, poly(A) tail lengths can be generated much longer than is possible using a template-encoded tail. mRNAs with longer poly(A) tails are expressed for longer periods of time in cells. This mRNA is suitable for use in transfection and microinjection experiments as well as *in vitro* translation systems. In applications where immunogenicity may be of concern, use of modified nucleotides during transcription and removal of dsRNA is advisable. CELLSCRIPT™'s INCOGNITO™ T7 mScript™ Complete Kits offer full mRNA synthesis using modified nucleotides and Min-Immune™ Gold enzymatic dsRNA reduction to less than 0.005% (LLOQ).

Standard CAR-T therapy workflow in oncology

CELLSCRIPT™'s *in vitro* transcription technologies have been cited in several publications[9, 10] regarding the generation of chimeric antigen receptor transcription surface proteins that bind to and program killer T-cells for recognition of tumor cells. Once programmed, T-cells induce destruction of antigen-containing tumor cells by release of cytokines. This action results in tumor cell damage and shrinkage with the hopeful clinical outcome that the lives of patients suffering from metastatic diseases will be prolonged. While expensive, CAR-T cell therapy has been shown to be another successful methodology for reduction and potential elimination of cancers. ■

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CELLSCRIPT™ technologies for CAR-T research mRNA production

Description	Catalog No.
T7 mScript™ Complete Standard mRNA Production System	MSCC250225
INCOGNITO™ T7 mScript™ Complete Ψ-mRNA Production System	IMCY250125
INCOGNITO™ T7 mScript™ Complete N1meΨ-mRNA Production System	IMCMY250225
T7 mScript™ Standard mRNA Production System V2	C-MSC11610 (10 RXN) C-MSC100625 (25RXN)
Min-Immune™ Gold dsRNA Removal Kit	MGDR250125
T7-FlashScribe™ Transcription Kit V2	C-ASF3507
ScriptCap™ Cap 1 Capping System	C-SCCS1710 (10 RXN) C-SCCS2250 (50 RXN)
A-Plus™ Poly(A) Polymerase Tailing Kit	C-PAP5104H

TempliAMP™ RCA Kit

Rolling Circle Amplification (RCA) is the isothermal game-changer that's revolutionizing how we amplify circular DNA constructs like plasmids.

- No thermal cycling required
- Easy scalability
- Works directly from bacterial colonies (no DNA extraction!)
- Ultra-high fidelity with Phi29 polymerase

Our TempliAMP™ RCA Kit lets you amplify DNA directly from colonies on agar plate, glycerol stocks and liquid cultures without the necessity of PCR.

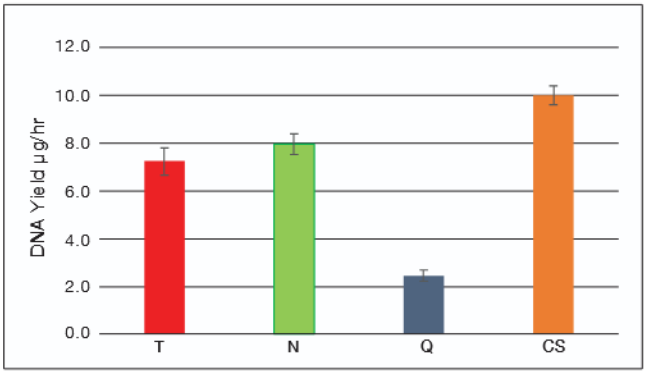


Figure 1. Comparison of DNA yields per hour (µg/hr) from TempliAMP™ RCA Kit against three commercial RCA Kits (T=Thermo Fisher, N=New England Biolabs, Q=QIAGEN). Input purified plasmid DNA was 5 ng for all kits. Highest yield per hour was obtained with TempliAMP™ RCA Kit.

Learn More
www.cellscript.com/TempliAMP

Application Note

TempliAMP™ RCA Kit

Performance analysis against commercial RCA Kits for DNA yield and workflow time

Daniel Gerhardt MS, Kari Weber PhD, Ronald Meis PhD, CELLSCRIPT™ 726 Post Road, Madison, WI 53713 USA

Introduction

Rolling Circle Amplification (RCA)[1-3] is an isothermal nucleic acid amplification technique often used to amplify circular DNA constructs such as plasmids. RCA utilizes phi29 DNA polymerase, which extends primers and continues to synthesize DNA around the circular template by displacing the previously synthesized DNA strand when encountered (Figure 1).

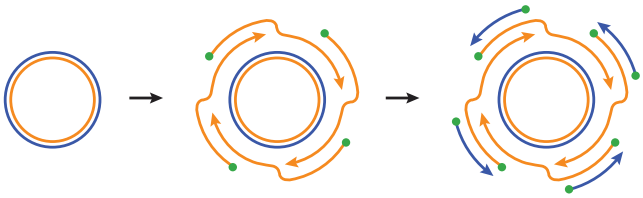


Figure 1: RCA DNA amplification process. Random primers (green dots) will anneal to multiple locations on both sense (blue) and anti-sense (orange) strands and amplify around the circle. This process will continue for the duration of the incubation, resulting in amplification of both strands of the target.

Key features of phi29 DNA polymerase include amplification at 30°C without thermal cycling, high processivity, strand displacement activity, and low error rate (1×10^{-6}-1×10^{-7}).[4-5] The method offers significant advantages, including:

1. **High sensitivity:** amplification from as little as 5 pg of DNA starting material
2. **Specificity:** phi29 DNA polymerase efficiency for a circular template reduces non-specific amplification
3. **Simplicity:** single-tube isothermal reaction requires minimal equipment

RCA is widely applicable for amplifying circular DNA. The TempliAMP™ RCA Kit from CELLSCRIPT™ offers a powerful solution for efficient and specific amplification of circular DNA, leveraging the high fidelity and strand displacement activity of phi29 DNA polymerase to minimize non-specific amplification. Traditional RCA methods, while effective, often suffer from long incubation times and inconsistent yields. In contrast, TempliAMP™ RCA Kit simplifies workflows by enabling direct amplification from bacterial colonies, glycerol stocks, or liquid cultures—eliminating the need for DNA extraction if desired. It also addresses common limitations such as low DNA yield, producing up to 30 µg of DNA from as little as 5 pg of input. The use of phosphorothioate-modified primers enhances primer stability and reduces degradation, while the high-fidelity phi29 polymerase ensures accurate and efficient replication. Together, these features reduce amplification time, increase specificity, and deliver consistent, high-quality results, making TempliAMP™ RCA Kit an ideal choice for plasmid preparation and sequencing applications.

In this application note, we examined the performance of CELLSCRIPT™'s TempliAMP™ RCA Kit against three commercially available RCA kits to demonstrate how TempliAMP™ RCA Kit provides consistent yields in less time while maintaining quality and specificity of the RCA DNA product.

Methods

Three other commercially available Rolling Circle amplification company products were evaluated in comparison to CELLSCRIPT™'s TempliAMP™ RCA Kit under standardized conditions: EquiPhi29™ DNA Amplification Kit (Thermo Fisher Scientific), phi29-XT RCA Kit (New England Biolabs) and REPLI-g® Midi Kit (QIAGEN). All reactions were performed in quadruplicate using a fixed input of 5 ng of plasmid DNA. RCA reactions were carried out following the respective kit instructions. Incubation times and temperatures were selected based on each manufacturers' recommended protocol: 4 hours at 30°C for the TempliAMP™ RCA Kit, 2 hours at 42°C for both the EquiPhi29™ DNA Amplification and phi29-XT RCA Kits, and 16 hours at 30°C for the REPLI-g® Midi Kit. After amplification, resultant amplified DNA was purified using AMPure® XP Bead-based Reagent (Beckman Coulter). The purified DNA was then digested with Xba I restriction enzyme (NEB), followed by a second round of purification using AMPure® XP beads. The plasmid used as template in the RCA reactions is ~8 kb and has a single Xba I restriction site, therefore amplification that is very specific will result in a single 8 kb band after digestion with Xba I.

Results

25 ng of each RCA product were visualized on a native 1% agarose gel. All products tested resulted in amplification of high molecular weight DNA (Figure 2A). Amplified DNA was digested overnight with Xba I and 25 ng was run on a native 1% agarose gel. The vast majority of Xba I digested RCA amplified DNA runs at the expected size (~8 kb), confirming that all kits produced the expected target and indicating successful and accurate amplification (Figure 2B).

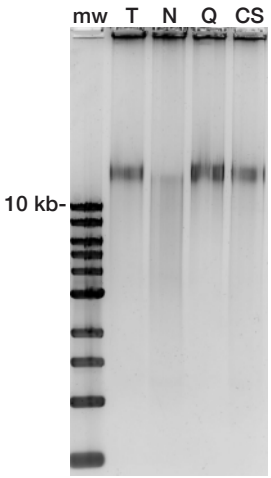
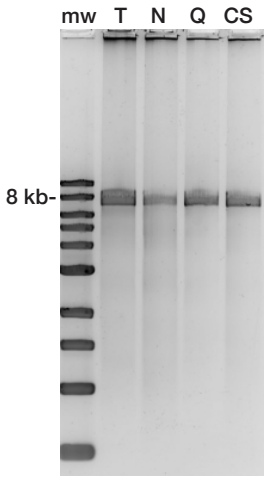


Figure 2A: Amplification of high molecular weight RCA DNA by products kits tested.



mw = 1 kb ladder (NEB)
T = EquiPhi29™ DNA Amplification Kit (Thermo Fisher)
N = phi29-XT RCA Kit (NEB)
Q = REPLI-g® Midi Kit (QIAGEN)
CS = TempliAMP™ RCA Kit (CELLSCRIPT™)

Figure 2B: RCA amplified DNA size following overnight Xba I digestion.

CELLSCRIPT™'s TempliAMP™ RCA Kit and QIAGEN's REPLI-g® Midi Kit produced the highest DNA yield compared to the other products tested (Figure 3A). When normalized against incubation time, CELLSCRIPT™'s TempliAMP™ RCA Kit generated more DNA template on a microgram per hour (µg/hr) basis compared to the other products tested (Figure 3B).

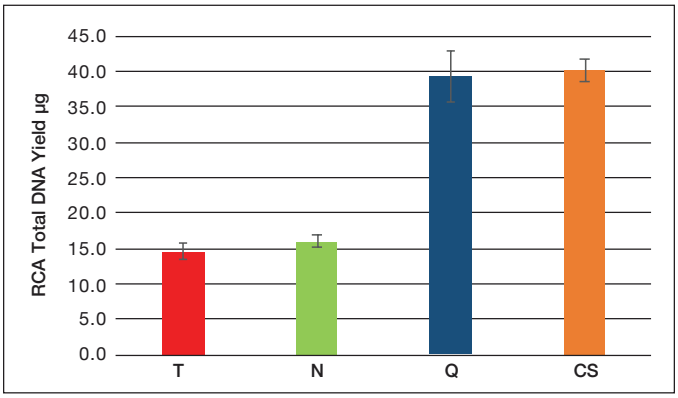


Figure 3A: RCA DNA yields in micrograms.

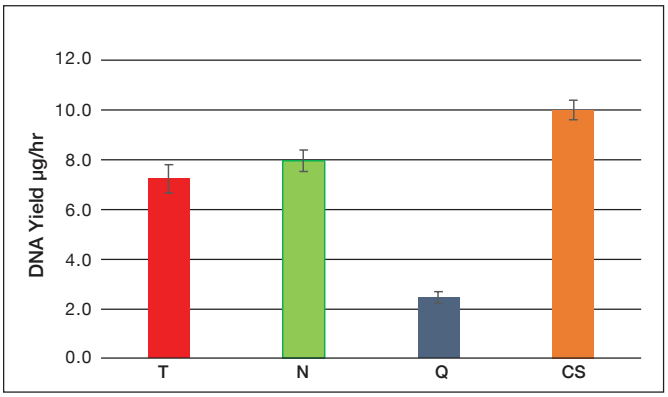


Figure 3B: RCA amplified DNA yield per hour of incubation.

Conclusion

The experimental results demonstrate that all four RCA products tested amplified DNA of comparable size, consistent with expected outcomes. Among the RCA company products tested, CELLSCRIPT™'s TempliAMP™ RCA Kit (Cat. No. TAR250325) delivers the most efficient yield-to-time ratio amongst the kits tested, underscoring its efficiency and suitability for time-sensitive workflows. Collectively, this work shows that while each of the evaluated RCA products can produce high-quality DNA suitable for downstream applications, CELLSCRIPT™'s TempliAMP™ RCA Kit offers a compelling combination of rapid processing and high specificity, making it a strong candidate for applications requiring efficient, precise and rapid DNA amplification. ■

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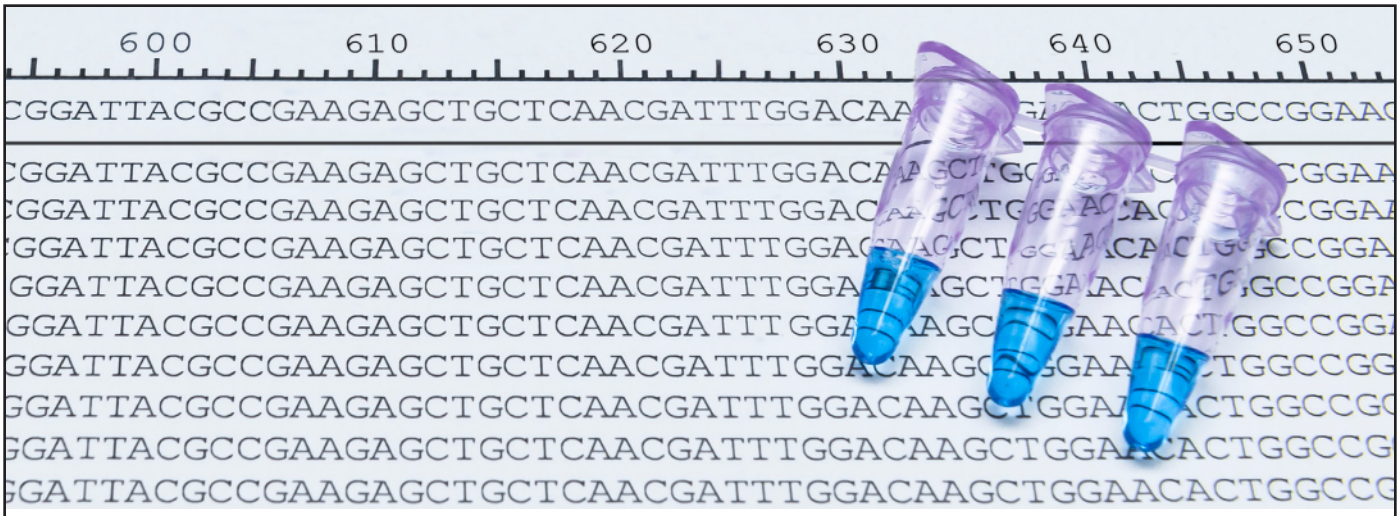
Trademarks

Did You Know?

- The Min-Immune™ Gold dsRNA Removal Kit can remove dsRNA to <0.005% (LLOQ) of the sample, lower than what can be achieved using engineered mutant T7 RNA polymerases.
- The INCOGNITO™ kits are designed for incorporation of modified nucleotides into synthesized RNA transcripts for immunogenicity reduction, a discovery made by Nobel Prize recipients Katalin Karikó and Drew Weismann and licensed exclusively to CELLSCRIPT™ for all fields of use.
- Strategic mRNA transcription with modified nucleosides paired with Min-Immune™ Gold dsRNA removal demonstrated a remarkable 887% improvement in protein expression while virtually eliminating immune activation.
- The EZ-QC™ mRNA Assay Kits produce results comparable to LC-MS while offering convenience, flexibility, and a cost-effective approach.
- The TempliAMP™ RCA Kit provides one of the highest efficiency DNA yield-to-reaction time ratios available in a kit.
- ScriptCap™ capping kits achieve virtually 100% capping of IVT RNA, a level unattainable using cap analogs.
- The A-Plus™ Poly(A) Polymerase Tailing Kit allows users to define their desired poly(A) tail length, even greater than 300 A's.
- Cap-Clip™ Acid Pyrophosphatase can decap any RNA 5′ cap structure containing a polyphosphate bridge, efficiently generating 5′-monophosphate RNA. It also converts uncapped 5′ triphosphate RNA into 5′-monophosphate RNA.
- The Adenylator™ Enzyme has no ligase activity, avoiding ligation byproducts like oligo concatemerization and circularization.



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The **Adenylator™ DNA/RNA 5′-Adenylation Kit** offers a catalytic, ligation-free method for adenylating oligos with no off-target byproducts (Figure 1) and 3X the output of competitor kits.

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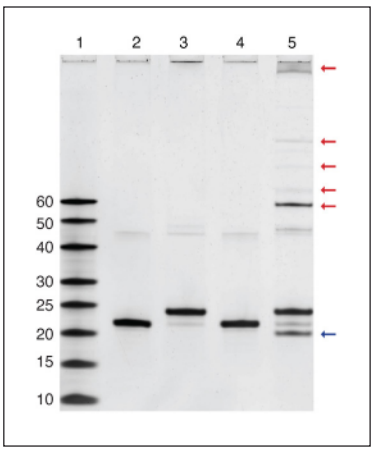
  No wasted byproducts
- **Increase workflow efficiency**
  3X the output of competitor kits
- **Stretch your budget for more experiments**
  Cost-effective alternative to pre-purchased oligos



1) IDT ssDNA 10/60 Ladder
2) Untreated (unadenylated) p-DNA Oligo (22-nt) in Adenylator™ reaction mix
3) Adenylator™ Enzyme Treated (37°C) p-DNA Oligo (23-nt)
4) Untreated (unadenylated) p-DNA Oligo (22-nt) in Mth RNA Ligase reaction mix
5) Mth RNA Ligase Treated (65°C) p-DNA Oligo (23-nt)

←) DNA Oligo Concatemers

←) Circular DNA Oligo

Figure 1. A 22-nt phosphorylated DNA oligo was adenylated using Adenylator™ Enzyme and Mth RNA Ligase and visualized on a polyacrylamide gel (20% acrylamide, 8 M urea, 1X TBE, stained with SYBR® Gold). The Mth RNA Ligase adenylated DNA oligo (lane 5) contains oligo concatemer (red arrows) and circularized oligo (blue arrow) ligation byproducts. Adenylation using Adenylator™ Enzyme (lane 3) cannot form substrate concatemers or circularize oligo substrates, resulting in adenylated oligos without byproducts.

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RNase R

Clean up circular RNA preps with efficient digestion of linear RNA.

1 µg RNase R digests 5 µg linear RNA in 30 minutes, leaving circRNA, lariat intron RNA and dsRNA intact.

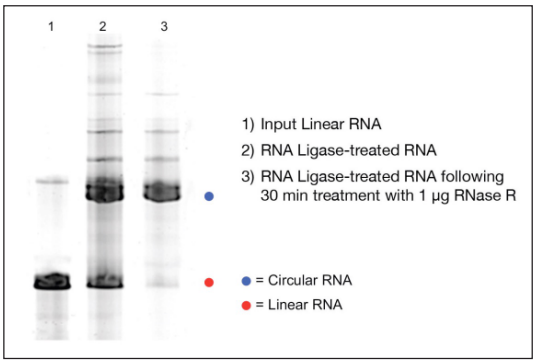

Figure 1. 5 µg of linear RNA was circularized with RNA Ligase to produce circular RNA (lane 2). The RNA ligation reaction product was treated with 1 µg of RNase R at 37°C for 30 minutes to enrich for circular RNAs (lane 3).

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Publication by application

CAR-T Cell Therapy

CD32B1, a versatile non-signaling antibody-binding scaffold for enhanced T cell adhesion to tumor stromal cognate antigens

CELLSCRIPT™ product used in research:

T7 mScript™ Standard mRNA Production System

Reference: Feigelson, S.W., et al., (2025) Front Immunol. 16:1398757

Preclinical efficacy of multi-targeting mRNA-based CAR T cell therapy in resection models of glioblastoma

CELLSCRIPT™ product used in research:

T7 mScript™ Standard mRNA Production System V2

Reference: Dagher, O.K., et al., (2025) Mol Ther Nucleic Acids. 36(3):102676

mRNA Vaccines – Cancer

An RNA vaccine against adrenomedullin reduces angiogenesis and tumor burden in a syngeneic metastatic melanoma mouse model

CELLSCRIPT™ products used in research:

T7-Scribe™ Standard RNA IVT Kit

INCOGNITO™ T7 5mC- & Ψ-RNA Transcription Kit

ScriptCap™ Cap 1 Capping System

Reference: Srdan Tadic, S. et al, (2025) Front Immunol.16:1604156

Enhancing the potency of 5T4 mRNA vaccine by CD70 mRNA-LNPs through ADCC and T cell boosting in prostate cancer therapy

CELLSCRIPT™ product used in research:

ScriptCap™ 2′-O-Methyltransferase Kit

Reference: Cao, F., et al., (2025) J Nanobiotechnology 23:523

mRNA Vaccines – Infectious Diseases

3-Deazaguanosine inhibits SARS-CoV-2 viral replication and reduces the risk of COVID-19 pneumonia in hamster

CELLSCRIPT™ product used in research:

ScriptCap™ m^{7}G Capping System

Reference: Tarashima, N.S, et al. (2025) iScience. 28(4):112140

Laminar fluid ejection device enables high yield and preservation of mRNA and SaRNA LNP formulations

CELLSCRIPT™ product used in research:

ScriptCap™ Cap 1 Capping System

Reference: Ho, C.H., et al., (2025) Sci Rep. 15:18507

A T cell-based ubiquitin-mediated mRNA vaccine provides cross-protection against H1N1 and B influenza viruses in mice

CELLSCRIPT™ products used in research:

T7-FlashScribe™ Transcription Kit

ScriptCap™ Cap 1 Capping System

Reference: Di, Y., et al., (2025) Curr Res Microb Sci. 9:100457

Neuropeptides

Flipped binding modes for the same agonist in closely related neuropeptide-gated ion channels

CELLSCRIPT™ product used in research:

T7-Scribe™ Standard RNA IVT Kit

Reference: Claereboudt, E.J.S., et al., (2025) Biophys J. 124(7):1049–1057

Oligonucleotide (ON)-based Therapy

G-quadruplex topologies determine the functional outcome of guanine-rich bioactive oligonucleotides

CELLSCRIPT™ product used in research:

ScriptCap™ m^{7}G Capping System

Reference: Kharel, P. et al., (2025) Nucleic Acids Res. 53(12)

Product RNA (pRNA) biology

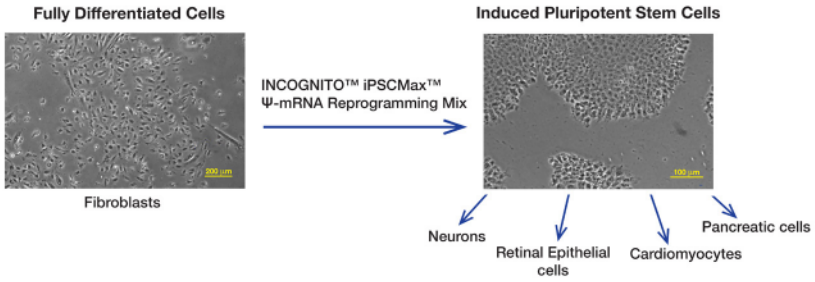
6S-1 pRNA 9-mers are a prominent length species during outgrowth of Bacillus subtilis cells from extended stationary phase

CELLSCRIPT™ product used in research:

A-Plus™ Poly(A) Polymerase Tailing Kit

Reference: Damm, K. et al., (2025) RNA Biol. 22(1):1–14

Maximize your fibroblast reprogramming with an ultra-low immunogenicity mRNA mix



INCOGNITO™ iPSCMax™ Ψ-mRNA Reprogramming Mix contains five Ψ-mRNA reprogramming factors optimized for ultra-low immunogenicity and highly-efficient fibroblast reprogramming.

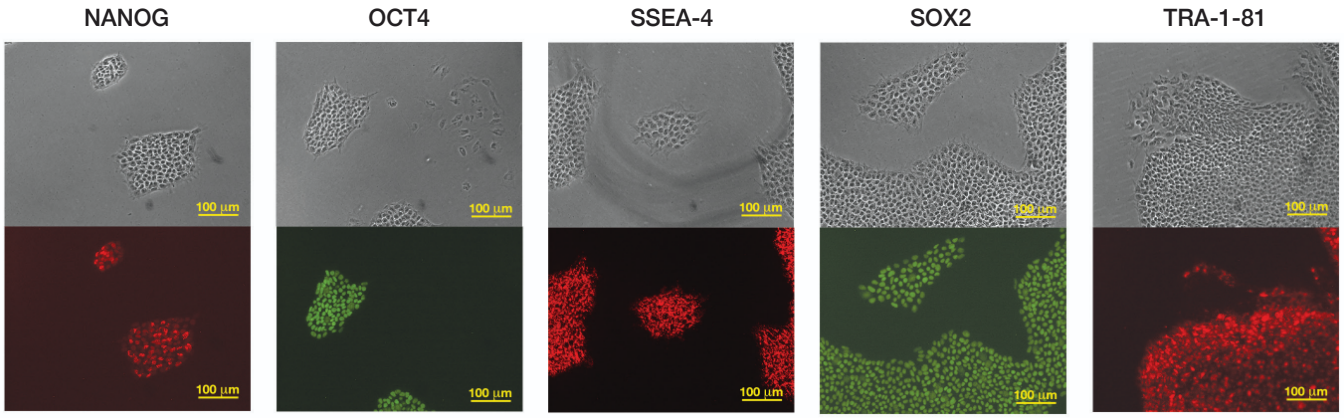


Figure 1. Cells stained for pluripotency markers using INCOGNITO™ iPSCMax™ Ψ-mRNA Reprogramming Mix.

- **Reduce innate immune activation** through pseudouridine-containing, virtually dsRNA-free mRNA.
- Reprogram fibroblasts into iPSCs with **as few as five transfections** and **get colonies within two weeks** (Figure 2).
- Rest assured with **peace of mind** that no residual vector will remain after reprogramming.
- Meet your research needs with either a mix for **6- or 12-wells**.

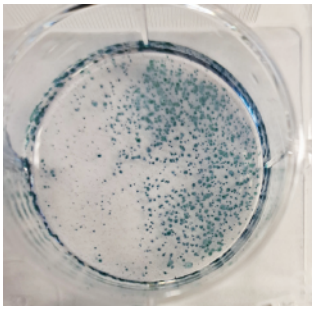


Figure 2. AP stain of colonies obtained using INCOGNITO™ iPSCMax™ Ψ-mRNA Reprogramming Mix.

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