ThermoFisher SCIENTIFIC

mRNA synthesis and purification

Scalable bead-based *in vitro* transcription and RNA purification for mRNA vaccine development and manufacturing

Keywords

Dynabeads magnetic separation products, solid-phase *in vitro* transcription, mRNA purification, mRNA vaccine production, generic capture purification, Dynabeads Streptavidin for *In Vitro* Transcription, template reuse, Dynabeads Carboxylic Acid for RNA purification, ancillary material for GMP production, recycling of beads, reduced antibiotics use

In this application note, we show:

- How Dynabeads magnetic separation products enable an easy, fast, and reliable method for manual synthesis and purification of mRNA
- How Dynabeads magnetic separation products enable flexible and highly scalable mRNA production
- How to obtain large amounts of mRNA from small amounts of plasmids or synthetic DNA templates and help reduce the manufacturing footprint
- How to reduce the use of antibiotics in template preparation by reducing the need for plasmids and recycling the template in *in vitro* transcription (IVT) reactions

Introduction

The world is in a new era of vaccine development, largely due to the success of mRNA vaccines in fighting the SARS-CoV-2 global crisis and the increasing occurrence of chronic and infectious diseases. However, vaccine production methods are not yet standardized, and the need for flexible and highly scalable production of mRNA remains urgent.

We have developed a new Invitrogen™ Dynabeads™ workflow for manufacturing mRNA, including a new magnetic-bead platform designed to support scalable GMP-grade synthesis and purification of mRNA. The workflow is easy, productive, flexible, and scalable. The same technology is easily adapted from research-scale to industrial volumes through a modular approach.

Quality, regulatory, and functional requirements are demonstrated, including optimal magnetic separation, handling, and performance with bench-scale systems and large-scale production in reactors.

invitrogen

Flexible and efficient mRNA synthesis and purification

Here we first describe advantages of template immobilization to magnetic beads. Then we present highly scalable workflows for *in vitro* transcription and mRNA purification with Invitrogen™ Dynabeads™ magnetic beads (Figure 1). The method can produce mRNA in amounts ranging from micrograms to grams with the same technology. By reusing the template 6 times, the total yield of mRNA is correspondingly increased without increasing the manufacturing footprint. Starting from a PCR-amplified template that is reusable, the amount of plasmid required and the need for antibiotics are reduced more than 10,000-fold. Intrinsic template purification occurs by template binding to Dynabeads magnetic separation beads. This step reduces the need for additional costly and time-consuming purification regimens and eliminates the risk of transfer of endotoxins and host DNA.

Easy, fast, and reliable mRNA production with flexible manual or automated workflow

The two Dynabeads products on the platform include streptavidin-conjugated beads for solid-phase *in vitro* transcription and carboxylic acid–activated beads for generic capture purification of mRNA.

mRNA production starts with a crude biotinylated PCR construct containing a linker region, a T7 promoter, and a target sequence. The biotinylated PCR product is purified by immobilizing it on Invitrogen™ Dynabeads™ Streptavidin for *In Vitro* Transcription (IVT) (Figure 1). The bead–DNA complexes are thoroughly washed, and no further purification of the PCR product is needed.

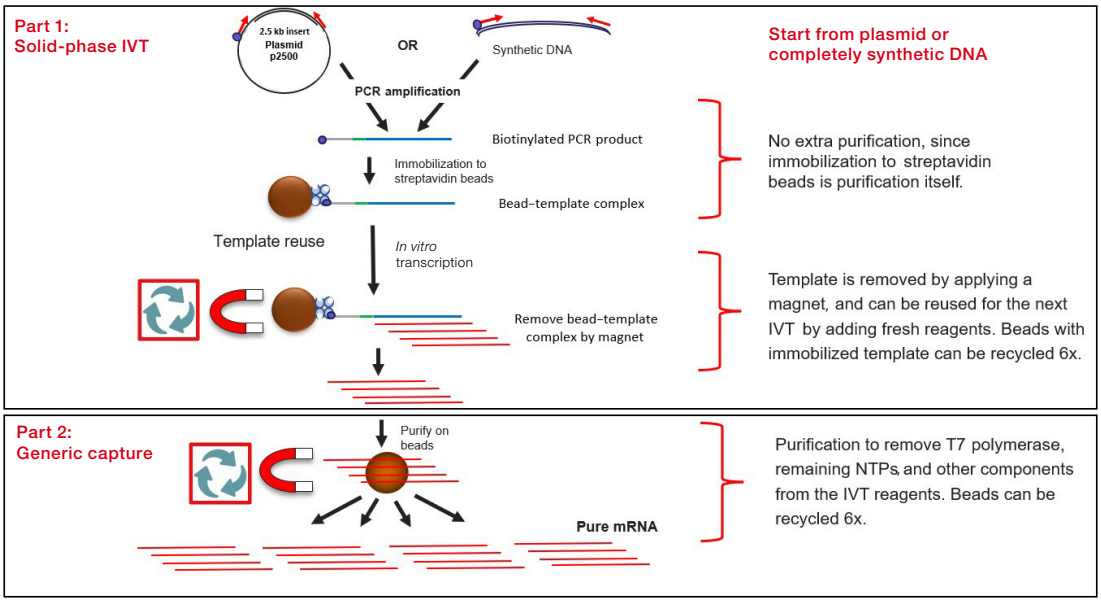


Figure 1. Solid-phase IVT for mRNA production starting from a plasmid or synthetic DNA followed by generic capture of the purified mRNA.

Add Dynabeads Streptavidin for IVT to the biotinylated template PCR product.

Mix and incubate for 30 min, place tube on DynaMag magnet for 1 min, and discard supernatant.

Wash bead–DNA complexes 6 times, place on DynaMag magnet, and discard supernatant between washes.

Resuspend the bead–DNA complexes in IVT master mix and incubate at 37°C for 1–3 hours. Remove template using a DynaMag magnet and transfer RNA to a clean tube. Reuse template in second IVT step.

Figure 2. Immobilization and IVT protocol for 100 µL to 1 L scale.

The bead–DNA complexes directly serve as templates for *in vitro* transcription when the beads are resuspended in the IVT master mix and incubated at 37°C for the desired time, typically 2–3 hours. When *in vitro* transcription is complete, the template is removed by applying an Invitrogen™ DynaMag™ magnet and transferring the synthesized RNA to a clean container. The bead–DNA complexes are then reused by adding fresh reagents and repeating the reaction. This recycling is efficient for at least 6 rounds of RNA synthesis (Figure 3A). The newly synthesized, template-free *in vitro* transcript is further purified by generic capture on Invitrogen™ Dynabeads™ Carboxylic Acid for RNA Purification.

Following the protocol in Figure 2, a 2.8 kb biotinylated PCR product containing the T7 promoter upstream of a 2.5 kb target sequence was immobilized on Dynabeads Streptavidin beads for IVT at a density of 2 µg/mg beads. The immobilization efficiency was more than 94% (data not shown). The bead–DNA complex was used as a template in IVT using 100 µL Invitrogen™ MEGAscript™ mix for 2 hours at 37°C. The immobilized template was reused in a total of 6 cycles of IVT by adding fresh reagents to the beads when the synthesized RNA was transferred to clean tubes. Each reaction resulted in an RNA yield of more than 4 mg/mL after 2 hours (Figure 3A). RNA integrity was analyzed on an Agilent™ 2100 Bioanalyzer™ Instrument, and the electropherogram overlays of the first, third, and sixth *in vitro* transcripts showed that integrity was nearly identical throughout 6 cycles of IVT (Figure 3B). An Applied Biosystems™ TaqMan™ qPCR assay specific for the DNA template upstream of the T7 promoter was used to quantify template leaching from the beads during the 6 cycles of IVT. The results showed that template leaching was low, with the highest amount occurring in the first IVT reaction (Figure 3C).

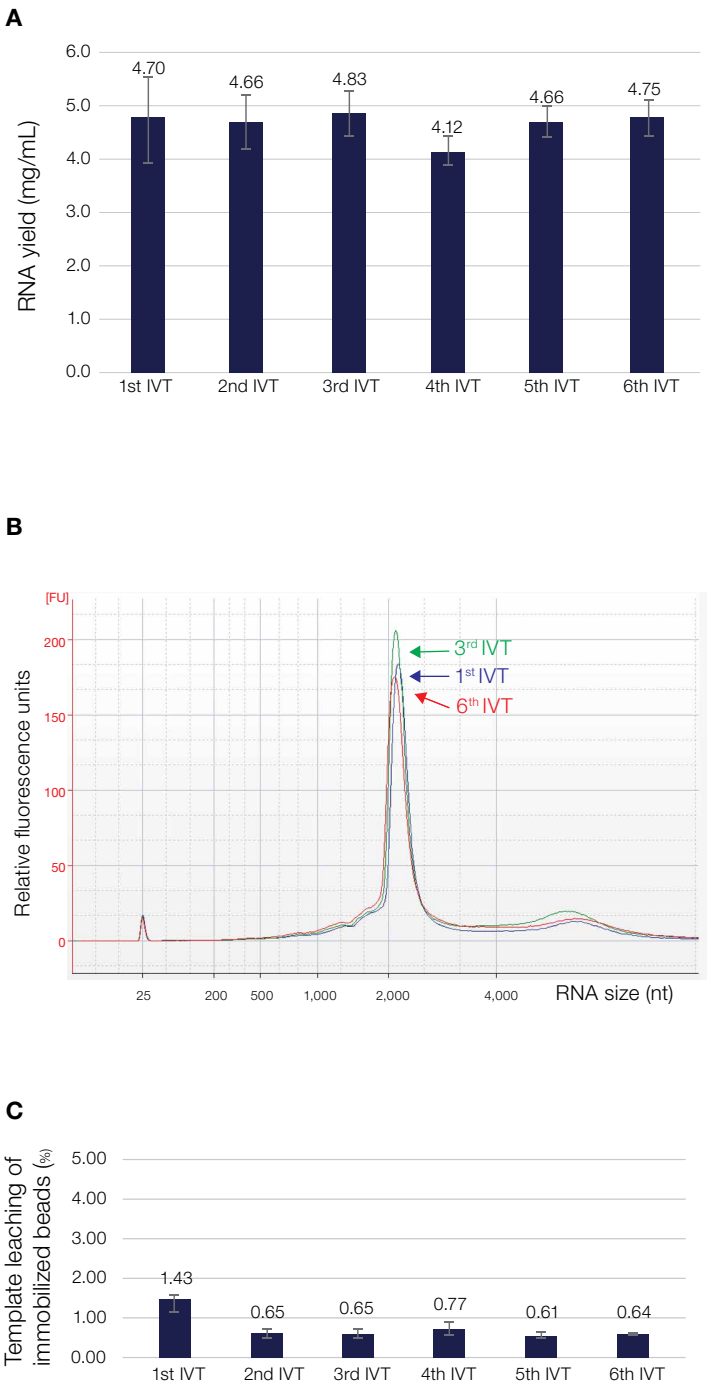


Figure 3. Example of template reuse in solid-phase *in vitro* transcription. (A) Reproducible yields of RNA from IVT in 6 subsequent cycles of template reuse. **(B)** Electropherogram overlays of RNA synthesized in the first, third, and sixth IVT cycles, showing nearly identical integrity of the synthesized RNA. **(C)** qPCR revealed that highest template leaching occurred in the first IVT step. This indicates less than 0.004% DNA contamination in the total crude RNA (data not shown).

Purification of crude IVT mRNA by generic capture on Dynabeads Carboxylic Acid for RNA Purification

The crude RNA was separated from the bead–DNA complexes and purified with Dynabeads Carboxylic Acid for RNA Purification in a simple bind-wash-elute protocol (Figure 4). An aliquot of the template-free mRNA was bound to the surfaces of the Dynabeads magnetic separation beads. The beads could be reused in up to 6 cycles of purification and yielded approximately 90% recovery in each cycle (Figure 5A). The integrity of the purified mRNA was analyzed on the 2100 Bioanalyzer Instrument.

Electropherograms of the purified mRNA from each cycle showed the overlaying of the crude *in vitro* transcript (Figure 5B), which indicated high and nearly identical integrity in each reuse of the beads. Samples of crude and purified mRNA were also analyzed by HPLC to show impurities had been removed (Figure 5C).

Add the template-free mRNA aliquot to Dynabeads Carboxylic Acid for RNA Purification and add 2 volumes of Invitrogen™ Dynabeads™ 1.5X RNA Binding Buffer.

Mix and incubate for 10 min at room temperature (RT), place tube on DynaMag magnet for 1 min, and discard supernatant.

Wash bead–mRNA complexes 3 times in 70% EtOH and place on DynaMag magnet for a brief drying period. Discard supernatant between washes.

Elute the purified mRNA by adding appropriate RNase-free elution buffer. Mix and incubate at RT for 5 minutes. Place tube on DynaMag magnet and transfer mRNA to a clean tube. The Dynabeads Carboxylic Acid for RNA Purification can be reused in multiple purification reactions.

Figure 4. Purification protocol.

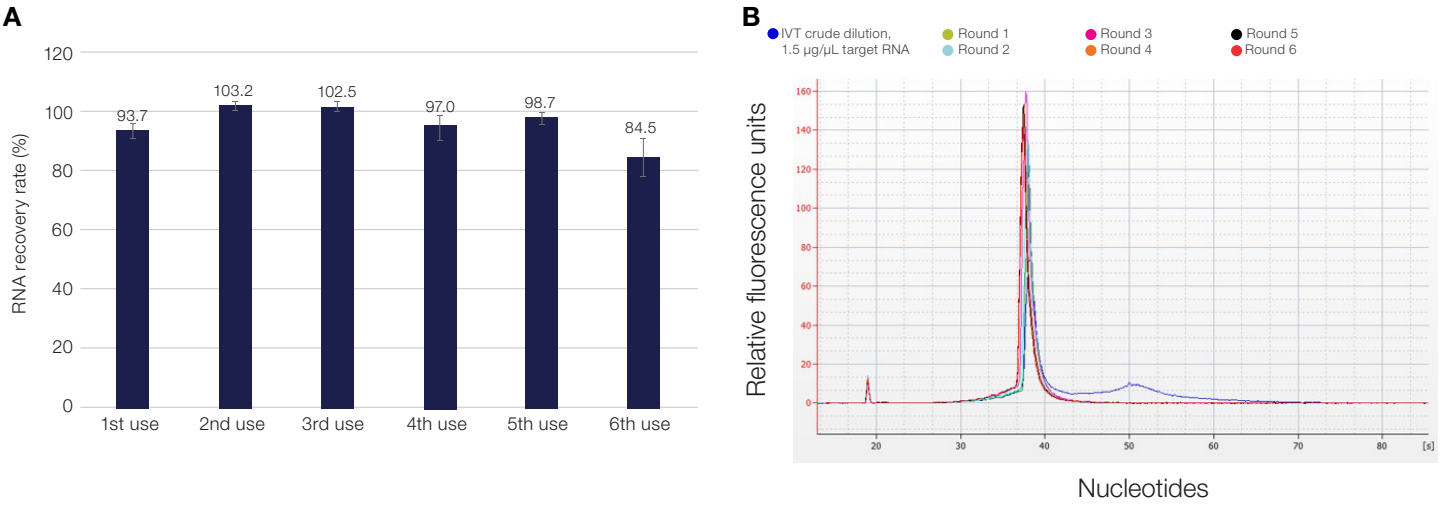


Figure 5. RNA purification by generic capture on Dynabeads Carboxylic Acid for RNA Purification. (A) mRNA recovery from crude *in vitro* transcript during recycling of the beads for mRNA purification showed consistently high yields. **(B)** Electropherogram overlay of crude mRNA and 6 aliquots of mRNA purified with the same recycled beads. The single blue peak represents the crude mRNA prior to the purification process. The data show that mRNA integrity is maintained throughout the purification steps. **(C)** HPLC chromatograms of crude and purified *in vitro* transcript showing that NTPs and enzymes were effectively removed by the generic capture step.

Scaling up the complete workflow

The complete Dynabeads workflow is highly scalable and performs equally well for volumes ranging from 100 µL to 1 L (Figure 6). Template immobilization was performed in a 1 L binding reaction in a glass reactor with an immobilization rate of almost 94% (Figure 6A). *In vitro* transcription was performed in the same 1 L glass reactor, and samples were taken every 30 minutes. The concentration of *in vitro* transcript increased over time (Figure 6B). The reaction was stopped after 2 hours at an mRNA concentration of 5.3 g/L in a 1 L volume. The template was removed by applying an external magnet to the reactor, and an aliquot of the template-free *in vitro* transcript was purified in a 1 L volume by directly upscaling the generic capture protocol. RNA recovery was approximately 100%, and the integrity was high, as indicated by the bioanalyzer results (Figure 6C).

Summary

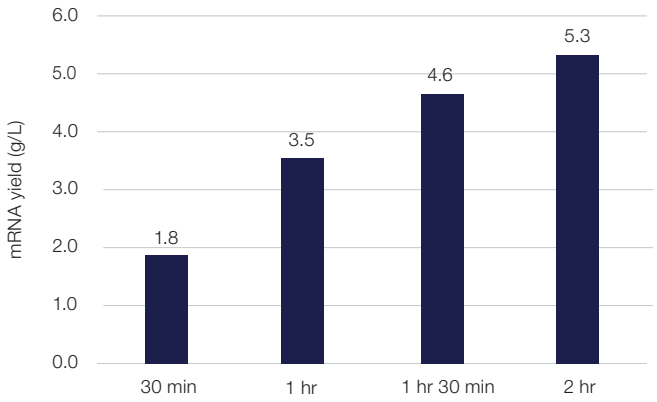
We have described a simple, flexible, and highly scalable magnetic bead–based method for the synthesis and purification of RNA for mRNA vaccine development and manufacturing. Synthesis of mRNA is performed by immobilizing the biotinylated template on Dynabeads Streptavidin for *In Vitro* Transcription. The template immobilized on the beads can be reused in IVT up to 6 times. Purification by generic capture of the *in vitro*–transcribed mRNA is performed by combining the crude IVT mix with Dynabeads Carboxylic Acid for RNA Purification. Purification is performed in a simple bind-wash-elute workflow that removes components remaining from the IVT reactions like enzymes and NTPs. Elution is done in the volume of choice.

Products manufactured under a mature quality system and ISO 13485 guidelines are designed to be suitable as ancillary materials for use in GMP manufacturing of therapeutics. Dynabeads magnetic separation products have been used by regulated markets for more than 35 years, including therapy-based markets. New Dynabeads magnetic separation products designed for the specific purpose of mRNA synthesis and purification demonstrate:

- A simple, automatable, flexible, and scalable workflow that enables reuse of DNA template
- >10,000 times more mRNA output than from plasmid preparation
- >10,000-fold reduction in antibiotic use relative to plasmid preparation
- Reduced number of bioprocessing steps
- Synthesis and purification steps that utilize technology suitable for diverse volume ranges (from µL to L scales)

A

B



C

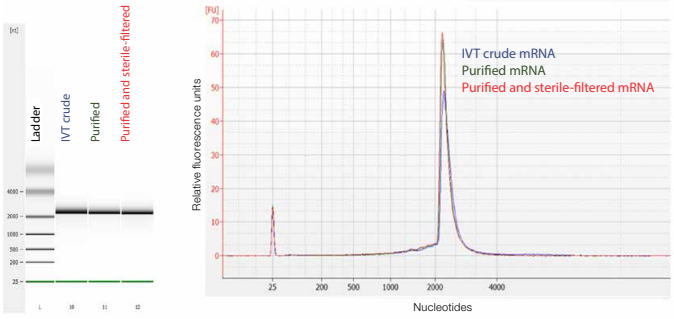


Figure 6. Scaling up the complete workflow to 1 liter in reactors. **(A)** Glass reactor showing the magnetic separation of beads after immobilization of 10 mg of the biotinylated PCR product to 1 g of Dynabeads Streptavidin for *In Vitro* Transcription, in a 1 L reaction volume. **(B)** mRNA yields after 30 minutes, 1 hour, 1.5 hours, and 2 hours of the IVT reaction. **(C)** Gel image and electropherogram showing the integrity of crude, purified, and purified and sterile-filtered IVT mRNA.

Ordering information

Product	Cat. No.
Dynabeads Streptavidin for *In Vitro* Transcription	49010D
Dynabeads Carboxylic Acid for RNA Purification	49020D
Dynabeads 1.5x RNA Binding Buffer	37035D
DynaMag-2 Magnet	12321D

Learn more at **thermofisher.com/dynabeadsmrna**

invitrogen