

## ENFINIA™ IVT READY DNA

# Frequently Asked Questions

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## What is ENFINIA IVT Ready DNA?

ENFINIA IVT Ready DNA is synthetic, linear, double-stranded DNA that can be directly utilized for *in vitro* transcription (IVT) to generate mRNA. ENFINIA templates contain an encoded poly(A) tail at the 3' end and can be immediately used for IVT without further processing.

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## How do I place an order for ENFINIA DNA?

New customers can use their work email to register for Elegen's [Order Submission Portal](#). Once an account is created, a user can click the "Order Now" button from the "Home" page, select ENFINIA IVT Ready DNA, and enter sequences individually or by uploading a file in .XLS, .CSV, .TSV, or FASTA format.

All entered sequences will be analyzed for synthesis feasibility based on sequence length and complexity (e.g., GC content, sequence repeats, and homopolymers) and according to submission criteria detailed in the [IVT Ready DNA Specifications and Submission Guidelines](#). If any of your sequences are rejected, a detailed reason is provided. You will be able to select a poly(A) tail for each sequence from a dropdown menu.

Once billing and shipping information is entered, users can proceed to checkout, where they'll be prompted to enter payment information and accept our terms and conditions. Users will also be asked to acknowledge our biosecurity terms, which comply with the Harmonized Screening Protocol created by the International Gene Synthesis Consortium.

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## What information do I need to order ENFINIA IVT Ready DNA?

To order ENFINIA IVT Ready DNA, you will need to provide DNA sequences that each include a promoter, 5' and 3' UTRs, and open reading frame (ORF). For sequences that pass our acceptance criteria, Customers will be able to select the poly(A) tail from a dropdown menu before submitting the order for production.

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## What yields of ENFINIA IVT Ready DNA are available?

Our standard offering of ENFINIA IVT Ready DNA is 10 µg. For larger amounts please contact your Elegen representative.

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## What lengths and complexities of ENFINIA IVT Ready DNA can be made?

Sequences of ENFINIA IVT Ready DNA ranging from 1 - 5.5 kb can be made (including the promoter, 5' and 3' UTR, and ORF). The poly(A) tail is not part of the length restriction. If you have a sequence shorter than 1 kb, you may add a random sequence of nucleotides upstream of the promoter in order to extend the length to 1 kb. Please note that this random sequence will not be transcribed to mRNA.

ENFINIA IVT Ready DNA is amenable to both standard and complex DNA constructs. Please refer to the [ENFINIA IVT Ready DNA Specifications and Submission Guidelines](#) for more information.

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**What poly(A) tails are available for ENFINIA IVT Ready DNA?**

We offer both continuous and segmented poly(A) tails. The segmented tails incorporate a 10 bp UGC linker (5'-GCATATGACT-3') to separate two deoxyadenosine homopolymers.<sup>1</sup>

Continuous: A70, A90, A100, A110, A120, A130

Segmented: A30-Linker-A70, A30-Linker-A90

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**What method do you use for adding the poly(A) tail to ENFINIA IVT Ready DNA?**

Poly(A) tails are added using a proprietary ligation-based method.

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**How do you ensure accurate poly(A) tail length?**

Elegen has taken steps to ensure poly(A) tail accuracy as part of quality assurance. Please see this [white paper](#) demonstrating accurate poly(A) tail length and low polydispersity of ENFINIA IVT Ready DNA templates relative to conventional plasmid templates from a major supplier.

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**Is ENFINIA IVT Ready DNA made in a cell-free manner?**

Yes, ENFINIA IVT Ready DNA sequences are made without the use of cells. Sequences are synthesized using a proprietary method to selectively amplify perfect sequences (customer sequence + poly(A) tail) without bacterial cloning.

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**What is the turnaround time for an ENFINIA IVT Ready order?**

The typical turnaround time of a standard complexity ENFINIA IVT Ready DNA sequence is 10 - 17 business days after an order has been confirmed. If your order is placed after 6 p.m. ET, production will begin the next business day (Monday through Friday). Turnaround times do not include shipping time, which may vary based on your location.

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**Does Elegen ship worldwide?**

We ship ENFINIA DNA products worldwide from our manufacturing locations in the U.S. If you have questions about our ability to ship to your location, please contact [customer.support@elegenbio.com](mailto:customer.support@elegenbio.com).

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**How can I pay for my Elegen order?**

We have flexible payment options for orders and accept purchase orders (POs) and blanket POs. We also accept Visa, MasterCard, AMEX, and Discover credit cards.

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**How is ENFINIA IVT Ready DNA shipped?**

ENFINIA IVT Ready DNA is shipped dry at ambient temperature in a 96-well microplate with one sequence per well.

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**Are there other limitations on sequences that can be submitted?**

Yes, ENFINIA IVT Ready DNA has restrictions on the sequences that can be submitted. Please refer to the [IVT Ready DNA Specifications and Submission Guidelines](#).

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**Is there a minimum order size for ENFINIA IVT Ready DNA?**

No, there is no minimum order size, but we may be able to provide a discount for large orders.

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**How should I resuspend and store ENFINIA IVT Ready DNA?**

ENFINIA IVT Ready DNA is dried down in 100% molecular grade water before shipment instead of a buffer to avoid excess salts. We recommend resuspending ENFINIA IVT Ready DNA in molecular grade water or TE buffer (10 mM Tris, 0.1 mM EDTA, pH 7.5). If performing *in vitro* transcription (IVT), please refer to the manufacturer's instructions for your application. We recommend keeping your DNA at -20°C for long term storage.

For more information on using IVT Ready DNA, please see the [IVT Ready DNA User Guide](#).

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**How do you measure the yield of ENFINIA IVT Ready DNA?**

DNA yield is reported as measured by Thermo Fisher Qubit 1X dsDNA HS assay (Q33231) using Elegen optimized protocols.

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**Does ENFINIA IVT Ready DNA contain any adapters or overhangs?**

Yes, each ENFINIA IVT Ready DNA sequence includes a 22 bp adapter on the 5' end (5'-GCGAGTCTTAGCCTGCGACGCT-3'). Please note that this adapter is upstream of the promoter and will not be transcribed to mRNA.

The poly(A) tail of ENFINIA IVT Ready DNA has a 3-T overhang on the 5' strand. For more information on IVT Ready DNA structure, please see the [IVT Ready DNA User Guide](#).

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**Is ENFINIA IVT Ready DNA sequence-verified?**

Yes, the customer-provided sequence (sequence without the poly(A) tail) is NGS-verified. Our current sequencing method cannot provide high-confidence measurements of homopolymers longer than 10 bp.

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**How do I use ENFINIA IVT Ready DNA to make mRNA?**

mRNA production is a common application of IVT. To ensure mRNA stability and enhanced translation efficiency, both a 5' cap and a poly(A) tail must be added.<sup>2</sup> Since ENFINIA IVT Ready DNA has an encoded poly(A) tail the polyadenylation step is not necessary. A 5' cap structure can be formed on IVT transcribed RNA either during or after transcription using a variety of cap analogs and enzymatic methods. Users should select an approach based on their application.

Various commercially available kits support IVT and capping.<sup>3</sup> We highly recommend consulting the information associated with these kits when considering the design elements to incorporate into your ENFINIA IVT Ready DNA sequence.

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**How does the mRNA yield from ENFINIA IVT Ready DNA compare to that of conventional plasmid templates?**

While mRNA yield is highly dependent on the IVT protocol used we found very comparable results using an IVT kit from a major vendor and early customers have observed similar results using their own protocols. Please see our flyer, [ENFINIA™ IVT Ready DNA: High-Quality Templates for Improved mRNA Yield and Purity](#), for more information.

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### What type of pre- and post-order support do you offer?

Customers can place an order unassisted using Elegen's order submission portal. Once you've registered as a user through this portal, you can access our Help Center, where the Customer Care Team is available to help you answer any questions you may have throughout the ordering process.

Once an order has been placed, a personal account manager will be in contact to ensure the order is received on time. For general inquiries involving our products or sales, please contact us at:

[customer.support@elegenbio.com](mailto:customer.support@elegenbio.com)

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### What information will I receive along with my ENFINIA IVT Ready DNA order?

A manifest will be emailed to you when your order is shipped. This document will contain the well location, yield, and QC status for each sequence.

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### How many attempts are made to synthesize ENFINIA IVT Ready DNA?

For most sequences, ENFINIA IVT Ready DNA can be successfully synthesized in a single production round. However, challenging sequences may require a second attempt. If a sequence cannot be produced after two attempts, it will be considered infeasible and you will not be charged.

In some cases, the full yield may not be achieved in a single production round. When this occurs, the partial product will be shipped immediately, followed by a second shipment containing the remaining material.

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### Can you provide a CoA for ENFINIA IVT Ready DNA?

At this time we do not provide a standard certificate of analysis with our shipments, but depending on your specific requirements we may be able to provide a suitable substitute. Please contact our customer support team at [customer.support@elegenbio.com](mailto:customer.support@elegenbio.com) for more information.

1. Chaudhary, N. 2021. *mRNA vaccines for infectious diseases: principles, delivery and clinical translation*.
2. New England Biolabs. *Minding your caps and Poly A tails – Strategies for synthesizing in vitro transcribed (IVT) mRNA*.
3. Example: *MEGAscript™ T7 Transcription Kit*



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