

ENFINIA™ IVT Ready DNA

Recommendations for Use

ENFINIA IVT READY DNA TEMPLATE STRUCTURE

ENFINIA IVT Ready DNA is a double-stranded DNA template for *in vitro* transcription (IVT) to RNA. Each sequence includes an adapter at the 5' end, positioned upstream of the promoter. This is followed by the customer-defined sequence, which includes a promoter, 5' untranslated region (UTR), open reading frame (ORF), and 3' UTR. A poly(A) tail is included at the 3' end.

- **Adapter**

The adapter sequence, 5'- GCGAGTCTTAGCCTGCGACGCT-3', will be added to the customer-defined sequence by Elegen to enable manufacturing. Please note that this sequence is upstream of the promoter and will not be transcribed to RNA.

- **Promoter**

The promoter sequence should be included in the customer-defined sequence. Use an appropriate promoter for *in vitro* transcription, such as the T7 RNA polymerase, which binds to and initiates transcription at the T7 promoter sequence (5'-TAATACGACTCACTATA-3').

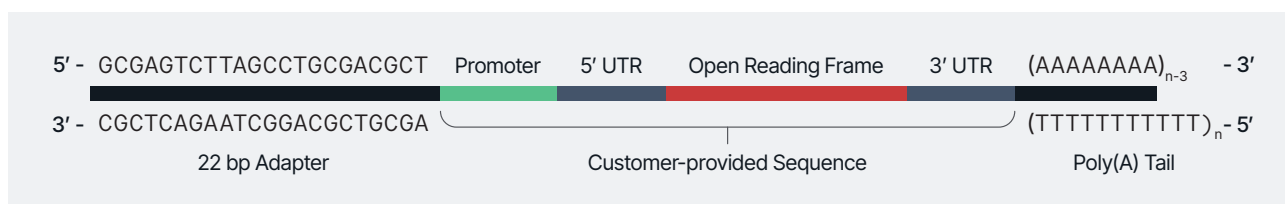
- **UTRs**

The 5' and 3' untranslated regions (UTRs) should be included in the customer-defined sequence. Sequences from alpha and beta globin genes are widely used because they are associated with high mRNA stability and translation efficiency.¹ For instance, the 5' UTR of the Pfizer-BioNTech COVID-19 vaccine (BNT162b2) is derived from the α -globin gene (HBA1).² However, it is common practice to screen for optimal 5' and 3' UTRs to identify and optimize effective sequences.

- **Poly(A) Tail**

A continuous or segmented poly(A) tail selected at the time of order will be added to the 3' end of the customer-defined sequence (see our [Specifications and Submission Guidelines](#)). Please note that there is a 3-T overhang on the 5' strand of all poly(A) tail options (see diagram below).

DIAGRAM OF *IN VITRO* READY DNA



SEQUENCE DESIGN FOR *IN VITRO* TRANSCRIPTION (IVT)

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.³ ENFINIA IVT Ready DNA has an encoded poly(A) tail, eliminating the need for an additional polyadenylation step. 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 specific to their application.

Various commercially available kits support IVT, capping, and polyadenylation of IVT RNA.⁴ We highly recommend consulting the information provided with these kits before designing your ENFINIA IVT Ready DNA sequence.

AMOUNT OF IVT READY DNA TO ORDER

When compared to linearized plasmid templates, a lower mass of ENFINIA IVT Ready DNA may be necessary for the *in vitro* transcription reaction. This likely results from a higher molarity of the ENFINIA template, which includes only the gene of interest and the necessary elements for transcription. However, we recommend optimizing the amount of template for the specific IVT kit and protocol used. An example comparison of mRNA yield resulting from ENFINIA IVT Ready DNA and linearized plasmid can be found here: [ENFINIA™ IVT Ready DNA: High-Quality Templates for Improved mRNA Yield and Purity](#).

RESUSPENSION

ENFINIA™ IVT Ready DNA is dried down in 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). As a general recommendation, always refer to the manufacturer's instructions for your specific application.

1. Reshetnikov, V. et al. [Untranslated Region Sequences and the Efficacy of mRNA Vaccines against Tuberculosis](#). *Int J Mol Sci*. 2024 Jan 10;25(2):888. doi: 10.3390/ijms25020888.
2. Kumari, M. et al. [A critical overview of current progress for COVID-19: development of vaccines, antiviral drugs, and therapeutic antibodies](#). *J Biomed Sci* 29, 68 (2022). doi: 10.1186/s12929-022-00852-9
3. New England Biolabs. [Minding your caps and Poly A tails – Strategies for synthesizing in vitro transcribed \(IVT\) mRNA](#).
4. Example: [MEGAscript™ T7 Transcription Kit](#)

