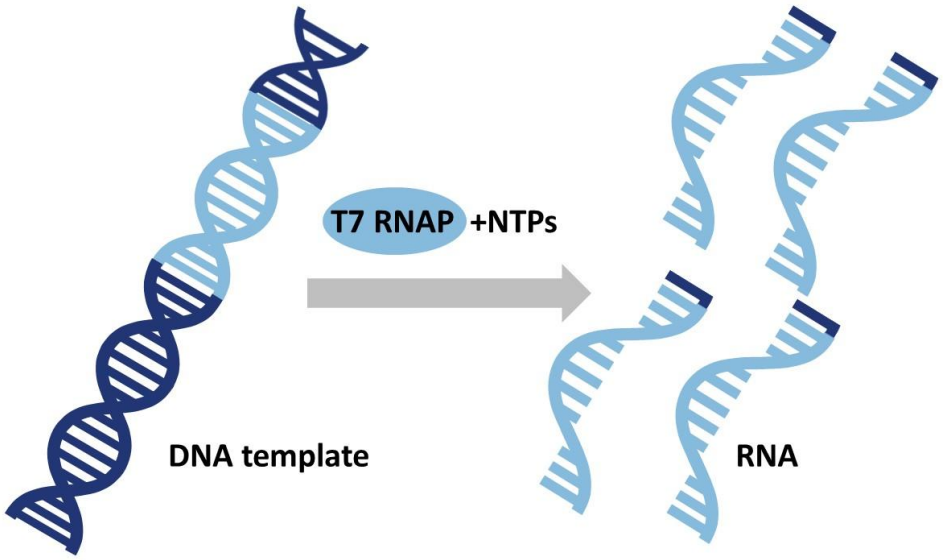
baseclick
DNA & RNA EXPERTS

User Manual

IVT High Performance T7 RNA Synthesis Kit



Version 1.0

Ordering Information

(for detailed kit content see **Table 1**)

Product Number	Product Name
BCK-RNA-IVT50	IVT High Performance T7 RNA Synthesis Kit

For References, FAQs and ordering please see online or contact us:

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Content

IVT High Performance T7 RNA Synthesis Kit

1. Introduction and Product Description

The baseclick *IVT High Performance T7 RNA Synthesis Kit* provides an efficient solution for producing large quantities of RNA by *in vitro* transcription (IVT) from DNA templates containing a T7 RNA polymerase promoter.

Designed for optimal performance, the kit enables high-yield RNA synthesis using 1 µg of linearized plasmid DNA as the template. Each kit contains reagents for 50 reactions, with a standard reaction volume of 20 µL. Depending on the template sequence, approximately 140 – 180 µg of RNA are generated in a single reaction, allowing the kit to produce a total yield of up to 9 mg of RNA.

This versatile kit supports the synthesis of capped RNA transcripts, the incorporation of modified nucleotides, in particular uridines. These features make it ideal for downstream applications, including post-synthetic RNA modification via click chemistry.

For research use only.

Please read the material safety data sheets (MSDS) provided for each product/component available online at www.baseclick.eu.

Literature Citation: When describing a procedure for publication using this product, please refer to it as the *IVT High Performance T7 RNA Synthesis Kit*.

2. Materials Provided with the Kit and Storage Conditions

Table 1: Kit components and storage conditions

Lid colour	Quantity	Component	Storage
Component A orange	50 µL	T7 RNA Polymerase Stock Solution	– 20 °C
Component B purple	28 µL	Pyrophosphatase Stock Solution	– 20 °C
Component C yellow	310 µL	5x Transcription Buffer	– 20 °C
Component D	80 µL	Cytidine triphosphate (CTP) 100 mM	– 20 °C
Component E	80 µL	Uridine triphosphate (UTP) 100 mM	– 20 °C
Component F	80 µL	Guanosine triphosphate (GTP) 100 mM	– 20 °C
Component G	80 µL	Adenosine triphosphate (ATP) 100 mM	– 20 °C
Component H blue	5 µg	Linear DNA Control Template 0.5 µg/µL	– 20 °C

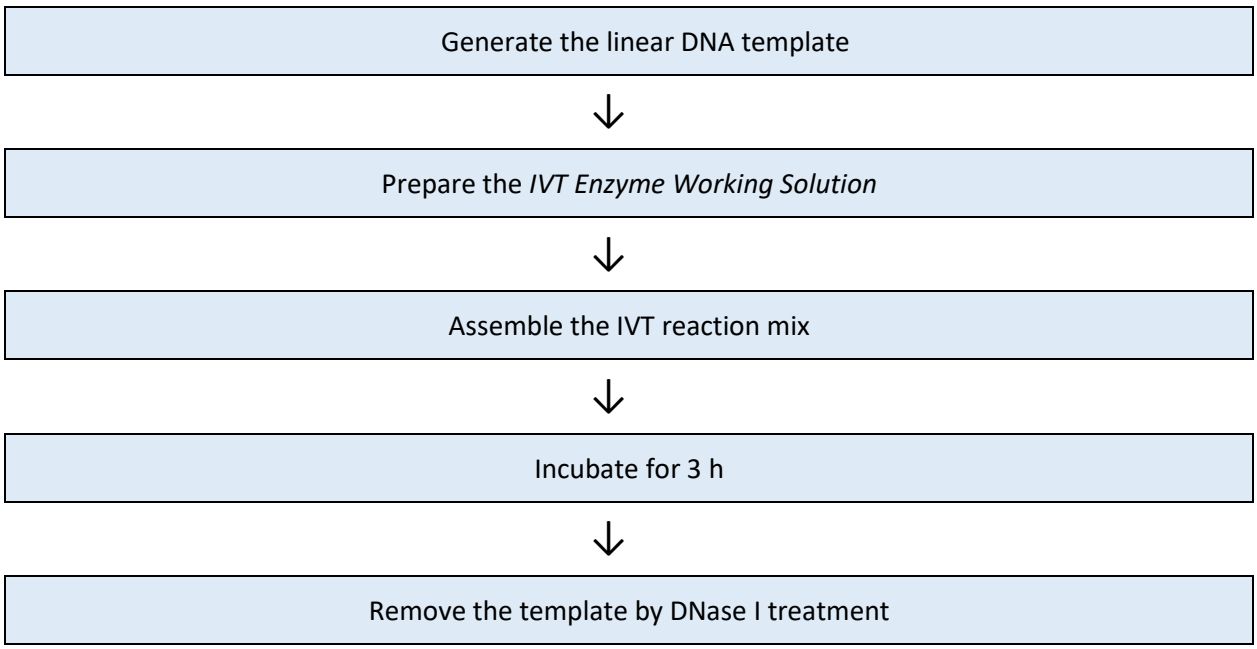
Note: The *5x Transcription Buffer* contains DTT to prevent RNA polymerase oxidation.

3. Materials and Equipment to be Supplied by the User

- A linearized template DNA containing a T7 promoter and the gene of interest
- Microcentrifuge tubes (RNase-free)
- RNase-free water (e.g. DEPC-treated water)
- RNase inhibitor
- Thermal mixer, thermocycler or water bath
- DNase I (RNase-free); Note: RNase-free is of fundamental importance
- Optional: Nanophotometer or other method for quantification of RNA transcripts
- Optional: Equipment for polyacrylamide or agarose gel electrophoresis

4. Workflow

Workflow scheme for RNA production by IVT



5. DNA Template

Double-stranded linear DNA is required as a template for *in vitro* transcription using the *IVT High Performance T7 RNA Synthesis Kit*. Suitable templates include linearized plasmids, PCR products, or cDNA, as long as the fragment contains a double-stranded T7 RNA polymerase promoter region (consensus sequence: TAATACGACTCACTATAGGG) in the correct orientation.

The poly(A) tail increases mRNA stability and translation efficiency in cells. Therefore, we recommend using a DNA template that includes a poly(A) tail region of minimum 30 nucleotides and for enhanced translation between 80 – 120 nucleotides. If the poly(A) tail is not present in the DNA template, it can be added post-transcriptionally using enzymes such as *E. coli* poly(A) polymerase and ATP (**BCT-42**).

6. *In Vitro* Transcription Protocol

6.1 Thaw the components:

- Thaw the *5x Transcription Buffer* (**yellow, C**) and allow it to reach room temperature.
- Thaw the *NTPs* (**colorless, D-G**) and keep them on ice.
- Keep *T7 RNA Polymerase Stock Solution* (**orange, A**) and *Pyrophosphatase Stock Solution* (**purple, B**) at -20 °C.

6.2 Prepare the IVT Enzyme Working Solution (EWS):

- Combine the following in a microcentrifuge tube on ice:
  - 6.6 µL of RNase-free water.
  - 2 µL of *5x Transcription Buffer* (**yellow, C**).
  - 0.5 µL of *Pyrophosphatase Stock Solution* (**purple, B**).
  - 0.93 µL of *T7 RNA Polymerase Stock Solution* (**orange, A**).
- Gently mix by tapping the side of the tube with your finger, then spin down briefly.

Notes:

- This preparation is sufficient for 5 reactions. For larger number of reactions, adjust the quantities accordingly.
- To minimize pipetting errors, scaling down this preparation is not recommended, even if fewer than five reactions are planned. This is not a concern, as the kit provides sufficient enzyme stock to prepare the described *EWS* setup for up to fifty reactions.
- **Important:** Always prepare the *IVT Enzyme Working Solution* fresh before setting up a reaction to prevent enzyme degradation.

6.3 Set up the IVT reaction

- Assemble the IVT reaction in a separate microcentrifuge tube at room temperature by sequentially adding the components listed in **Table 2** below. For larger reaction numbers, adjust the quantities accordingly.

Notes:

- A minimum DNA concentration of 135 ng/µL is required to add 1 µg of linear template DNA to the reaction mix.
- For convenience, you can combine equal volumes of the four 100 mM nucleoside triphosphate (NTP) solutions to create a 25 mM NTP stock solution for each nucleotide.

Table 2: IVT reaction assembly in order of addition

Lid color	Reaction component	Amount [µL]	Final concentration
---	RNase-free water	7.5 µL - X µL	---
yellow, C	5x Transcription Buffer	4 µL	1x
colorless, D	CTP 100 mM	1.5 µL	7.5 mM
colorless, E	GTP 100 mM	1.5 µL	7.5 mM
colorless, F	UTP 100 mM	1.5 µL	7.5 mM
colorless, G	ATP 100 mM	1.5 µL	7.5 mM
---	Linear DNA template 1 µg	X µL	1 µg
---	RNase Inhibitor 40 U/µL	0.5 µL	1 U/µL
---	IVT Enzyme Working Solution	2 µL	---
---	**Total Reaction Volume**	**20 µL**	**---**

6.4 Incubate at 37 °C:

- Mix the reaction gently, briefly spin down, and incubate at 37 °C for at least 3 hours.

Note:

- Avoid freezing the transcription reaction after incubation. Proceed directly to DNase I treatment or nucleotide removal.

6.5 Remove template DNA by DNase I treatment:

- Add 4 U of DNase I, RNase-free (not supplied in the kit) to the reaction to remove template DNA.
- Mix thoroughly and incubate for 15 minutes at 37 °C.

6.6 RNA Purification

Purification of RNA after transcription is essential to remove excess nucleotides and enzymes. Spin column methods, such as the *Monarch® Spin RNA Cleanup Kit* (NEB) or the *RNA Clean & Concentrator® Kit* (Zymo Research), provide fast and reliable purification. However, all other types of RNA purification can be used. Purified RNA is suitable for a wide range of downstream applications. Alternatively, gel purification or phenol/chloroform extraction followed by ethanol precipitation can also be used to obtain high-quality RNA.

Important: For certain downstream applications of RNA transcripts, particularly those involving copper-catalyzed click chemistry, avoid elution buffers from spin column kits (often containing EDTA, a chelating ligand for copper). Instead, use RNase-free water for elution to ensure click reaction performance.

6.7 Determining RNA Concentration and Quality

After removal of the DNA template and unincorporated nucleotides, the RNA concentration can be easily determined by measuring the ultraviolet (UV) absorbance at 260 nm (A260) with a nanophotometer. One unit of A260 corresponds to approximately 40 ng/µL of RNA.

To evaluate the quality of the RNA transcript, we recommend denaturing gel electrophoresis. This method allows verification of RNA integrity and size. By comparing the RNA transcript with a RNA ladder, the transcript size can be confirmed.
Tip: High-quality RNA will appear as distinct, sharp bands on the gel, whereas smearing indicates degradation. To prevent RNA degradation, ensure that all equipment and reagents for quality analysis are RNase-free.

6.8 IVT Performance Evaluation

This kit includes a linearized plasmid control template (**blue, H**) encoding mRNA for the mScarlet protein (0.5 µg/µL). Using the control template in the IVT reaction typically yields approximately 90 µg of RNA, enabling the evaluation of RNA yield and integrity.

If you experience any issues with the control template, please feel free to contact us directly for assistance at support@baseclick.eu.

7. Synthesis of Capped and 5´-Modified RNA Transcripts

In eukaryotes, most mRNAs contain a 5'-cap structure, m^7G(5')ppp(5')G, essential for recruiting translation initiation factors and enhancing mRNA stability. This capping can be performed enzymatically after transcription, a method that requires additional reactions and purification steps, which increases complexity and processing time.

Alternatively, synthetic cap analogues such as CleanCap®-AG (**BCT-38**) or ARCA (**BCT-24**) can be incorporated directly during *in vitro* transcription. mRNAs with these caps exhibit stability and translation efficiency comparable to natural mRNAs. The *IVT High Performance T7 RNA Synthesis Kit* supports this approach. Follow the manufacturer's instructions for optimal yields.

baseclick also offers azide- (**BCT-39**) and alkyne-modified (**BCT-41**) cap analogues, allowing efficient 5'-RNA modification with a variety of moieties via click chemistry. Furthermore, an amine-containing cap analogue for NHS ester modification is also available (**BCT-40**). All baseclick cap analogues can be seamlessly incorporated into RNA using the *IVT High Performance T7 RNA Synthesis Kit*. Detailed instructions can be found in the respective user manuals (see also baseclick's webpage www.baseclick.eu).

8. IVT Reactions with Modified Nucleotides

Synthesis of Pseudouridine-, *N*1-Methylpseudouridine, or 5'-Ethynyl Uridine-Containing RNA

The *IVT High Performance T7 RNA Synthesis Kit* is fully compatible with the production of RNA transcripts containing stabilising UTPs such as pseudouridine, *N*1-methylpseudouridine, or, for longer protein expression, 5-ethynyl uridine (**BCN-003**). No modifications to the IVT protocol are required, and the ratio of *N*1-methylpseudouridine triphosphate (**BCT-37**), pseudouridine triphosphate (**BCT-23**), or 5-ethynyl uridine triphosphate (**BCT-07**) to natural NTPs can be freely chosen (ranging from 100% replacement of uridine to 1% replacement). The expected RNA yields are equivalent to those of unmodified RNA.

Incorporation of Clickable Nucleotides for Versatile RNA Conjugation

baseclick offers a broad range of alkyne- and azide-modified ribonucleotides for post-synthetic RNA modification using click chemistry. These and other modified nucleotides can be efficiently incorporated into RNA using the *IVT High Performance T7 RNA Synthesis Kit*. To achieve this, the standard IVT protocol must be adapted as described and recommended by the seller.

Please note that the incorporation of e.g. bulky or dye-labelled ribonucleotides can reduce transcription efficiency. In addition, RNA transcripts containing modified nucleotides will exhibit reduced electrophoretic mobility due to their increased molecular weight.

9. Troubleshooting Guide

If you encounter problems that are not covered in this section, please feel free to contact us at support@baseclick.eu.

Low RNA Yield

1. **RNase Contamination**

   RNase can degrade RNA resulting in reduced yields. To prevent this, ensure that all solutions (not provided in the kit) are prepared in water treated with 0.1 % DEPC to inactivate RNases. Always use RNase-free equipment and reagents.

2. **Reduced RNA Polymerase Activity**

   If the RNA polymerase is not functioning optimally, it can result in reduced transcription efficiency. You can test the activity of the enzyme by performing a control transcription reaction using the included control template (see **chapter 6.8**).

3. **Precipitation of DNA Template**

   DNA precipitation may occur due to spermidine present in the *5x Transcription Buffer*. To avoid this, ensure that the reaction components are assembled at room temperature and in the correct order, as specified in the protocol.

4. **Excessive NaCl Concentration**

   High concentrations of NaCl (greater than 30 mM) can inhibit RNA polymerase activity, potentially reducing efficiency by up to 50%. If the template DNA has been precipitated with NaCl, desalting may be necessary. This can be achieved by column chromatography, followed by reprecipitation with an alternative salt. After reprecipitation, wash the DNA pellet 1 – 2 times with 70% ethanol to remove residual salts.

Incomplete RNA Synthesis

1. **Premature Termination of RNA Synthesis**

   If RNA synthesis terminates before completing, try reducing the incubation temperature from 37 °C to 30 °C. In some cases, this adjustment can improve the production of full-length transcripts.

2. **Alternative Polymerase Strategy**

   If premature termination persists, consider subcloning the transcript sequence of interest into a different vector that uses an alternative RNA polymerase. Some sequences may be recognized as terminators by one polymerase but not by another, potentially improving transcription efficiency.

Transcripts Larger than Expected

1. **Presence of Nonlinearized Plasmid**

   If larger-than-expected transcripts are observed, this may be due to the presence of uncut (nonlinearized) plasmid in the reaction. Run the template sample on a gel to check for undigested vector. If undigested plasmid is detected, redigest the sample with the appropriate restriction enzyme to ensure complete linearization.

2. **Protruding 3'-Termini on the DNA Template**

   When using a restriction enzyme that produces 3'-overhangs, transcription may result in unusually long RNA molecules. This occurs because transcription can initiate at the overhanging end of the template. To avoid this, consider using a restriction enzyme that produces 5´-overhangs or blunt ends.