# T7 High Yield RNA Synthesis Kit FS-RT-30220

T7 High Yield RNA Synthesis Kit is a flexible kit for in vitro transcription of RNA using T7 RNA polymerase. Enables the substitution of NTPs for labeling and incorporation of modified bases.

The kit is suitable for synthesis of high yield RNA transcripts and for incorporation of modified nucleotides to obtain biotin labeled, dye labeled or capped RNA. The kit is also capable of synthesizing high specific activity radiolabeled RNA probes.

RNA synthesized from the kit is suitable for many applications including RNA structure and function studies, ribozyme biochemistry, probes for RNase protection assays and hybridization based blots, anti-sense RNA and RNAi experiments, microarray analysis, microinjection, and in vitro translation and RNA vaccines.

The T7 Enzyme Mix in the kit contains RNase inhibitors and inorganic pyrophosphatase, enabling a yield of at least **150 µg of RNA per reaction** from 1 µg of DNA template.

| Kit Components ( 50 reactions) | Size |
|---|---|
| T7 RNA Enzyme Mix | 100 µL |
| T7 Transcription Buffer (10x) | 100 µL |
| ATP (100 mM) | 100 µL |
| UTP (100 mM) | 100 µL |
| GTP (100 mM) | 100 µL |
| CTP (100 mM) | 100 µL |
| Control Template (0.5 µg/µL) | 10 µL |
| DNase I, RNase-free | 100 µL |

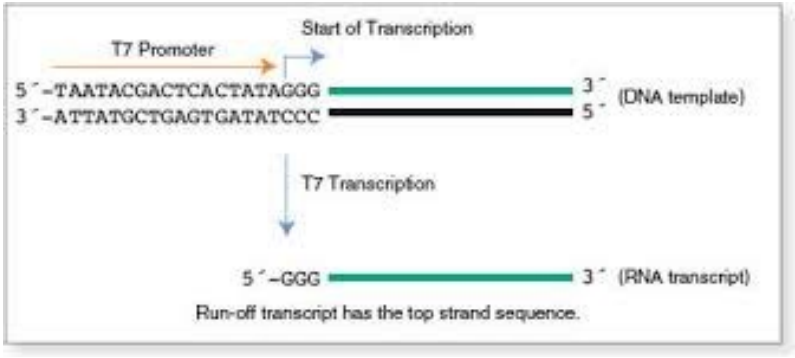
The kit contains sufficient reagents for 50 Reactions of 20 µl each

**Note:**

1.Prevent RNase contamination: When using this kit, wear a lab coat, disposable latex gloves, disposable masks, and use RNase-free consumables.

2.Template selection: It is recommended to purify the template before in vitro transcription to prevent contamination from RNase, protein, RNA, and salts.

3.Capped or modified RNA synthesis: If capped RNA is needed, prepare Cap Analog separately. If synthesizing labeled RNA (e.g., biotin, digoxigenin, FITC) or RNA with special modifications, the corresponding modified NTP needs to be prepared additionally.




**1. Figure 1** illustrates the minimal T7 promoter sequence and the start of transcription as well as a run-off transcript after T7 transcription

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# Operation Description

## 1. *DNA Template Preparation*

Linearized plasmid with a T7 promoter or a PCR amplification product can serve as in vitro transcription template. The template can be dissolved with TE buffer Nuclease-Free Water, with a recommended concentration of 0.5-1 μg/μL.

T7 promoter sequence: TAATACGACTCACTATAN*
Note: N* represents the first base of RNA transcription, usually G. If co-transcription occurs, the cap analog determines the first nucleotide.

### 1.1 *Plasmid Templates*

Plasmids containing T7 promoters can be used as transcription templates. However, plasmid linearization and purity affect transcription yield and RNA integrity. Circular plasmids can produce RNA of different lengths due to inefficient termination. In order to obtain a specific RNA length, plasmid must be fully linearized. The selected restriction enzyme should cut immediately downstream of the coding strand and should not have recognition sites within the coding region. The restriction enzyme should be selected to be able to generate blunt ends or 5´-overhangs.
Note: Purify linearized plasmids before transcription to remove enzyme, protein, and salt contaminants.

### 1.2 *PCR Templates*

PCR products containing T7 RNA Polymerase promoter in the correct orientation can be transcribed. The T7 promoter sequence is added to the 5' end of the primer upstream of the coding strand, and the DNA template containing the T7 promoter is amplified using high-fidelity enzyme and subsequently transcribed. Although crude PCR products can be used as templates, purification improves RNA yield.
Note: Verify PCR products by gel electrophoresis to confirm purity and concentration. It is recommended to use 2-5 μL of PCR product in a 20 μL in vitro transcription reaction.

## 2. *In Vitro Transcription*

2.1 Choose one of the following transcription reaction setups based on the desired RNA product. The recommended DNA template amount is 0.5-2 μg, with 1 μg used for initial experiments.

Note: Prepare the reaction system on ice, adding Nuclease-Free Water first, followed by ATP/CTP/GTP/UTP.

**1.Standard RNA Synthesis**

| Component | Volume | Final concentration |
|---|---|---|
| 10X T7 Transcription Buffer | 2 μL | 1X |
| ATP (100 mM) | 2 μL | 10 mM |
| CTP (100 mM) | 2 μL | 10 mM |
| GTP (100 mM) | 2 μL | 10 mM |
| UTP (100 mM) | 2 μL | 10 mM |
| T7 Enzyme Mix | 2 μL | / |
| Template DNA | x μL | 50 ng/μL |
| Nuclease-Free Water | up to 20 μL | / |

**2) RNA Synthesis with Modified Nucleotides**

| Component | Volume | **Final concentration** |
|---|---|---|
| 10X T7 Transcription Buffer | 2 μL | 1X |
| ATP (100 mM) | 2 μL | 10 mM |
| CTP (100 mM) | 2 μL | 10 mM |
| GTP (100 mM) | 2 μL | 10 mM |
| UTP (100 mM) | 1.5 μL | 7.5 mM |
| Modified UTP (50 mM)* | 1 μL | 2.5 mM |
| T7 Enzyme Mix | 2 μL | / |
| Template DNA | x μL | 50 ng/μL |
| **Nuclease-Free Water** | **up to 20 μL** | **/** |

*** Note: Biotin/fluorescein/digoxin or aminoallyl modified UTP is suitable for this system, and the transcription yield of modified UTP will be lower than that of unmodified UTP. This system takes modified UTP as an example, if other modified NTP substrates are used, please refer to the ratio of UTP and Modified UTP to prepare the reaction system.**

## 3. *Capped RNA Synthesis*

| Component | Volume | **Final concentration** |
|---|---|---|
| 10X T7 Transcription Buffer | 2 μL | 1X |
| ATP (100 mM) | 2 μL | 10 mM |
| CTP (100 mM) | 2 μL | 10 mM |
| UTP (100 mM) | 2 μL | 10 mM |
| GTP (100 mM) | 0.4 μL | 2 mM |
| Cap Analog (40 mM)* | 4 μL | 8 mM |
| T7 Enzyme Mix | 2 μL | / |
| Template DNA | 1 μg | 50 ng/μL |
| **Nuclease-Free Water** | **up to 20 μL** | **/** |

*** Note: The cap analog used here is m7(3'-O-methyl)-G(5')ppp(5')G (also known as ARCA) as an example, if use other cap analog, adjust the GTP-to-cap analog ratio accordingly.**

2.2 Mix thoroughly and centrifuge briefly, and incubate at 37°C for 2 hours. If the transcript length is < 100 nt, the reaction time can be extended to 3-16 hours.

2.3 After the reaction, add 2 μL of DNase I, RNase-free per μg of Template DNA to the product and incubated at 37℃ for 15 minutes to remove the DNA template.

2.4 The transcribed RNA can be purified and used for downstream experiments or stored at -80°C for future use.

## 3. *Transcription of The Control Template*

The control template provided with this kit is a linear DNA fragment containing a T7 promoter, with a transcript size of approximately 4000 nt. In the recommended standard RNA synthesis reaction, a minimum of 150 μg of RNA can be obtained by reacting at 37 °C for 2 h using 1 μg of control template DNA.

## 4. *Purification of Synthesized RNA*

The transcribed RNA can be purified using phenol/chloroform extraction, column purification, magnetic bead method, or lithium chloride precipitation to remove proteins and free nucleotides. If the size of the RNA product is well-defined, gel recovery purification is recommended for purification. After electrophoresis to assess the purified RNA, it can then be used for downstream experiments or stored at -80°C.

### 1) *Phenol/chloroform purification*

Phenol/chloroform extraction can remove proteins and most of the free nucleotides. The steps are as follows:

a. Dilute the product to 135 μL by adding 115 μL of Nuclease-Free Water.Then, add 15 μL of 3 M sodium acetate (pH 5.2) and mix thoroughly.
b. Add 150 μL of a 1:1 phenol/chloroform mixture for extraction. Centrifuged at maximum speed (≥ 12000 rpm) for 5 minutes at room temperature. Transfer the upper aqueous phase to a new RNase-free EP tube.
c. Add an equal volume of chloroform and perform two additional extractions. Collect the upper aqueous phase, and transfer it to a new RNase-free EP tube.
d. Add 2 volumes of absolute ethanol to precipitate the RNA. Mix well and place at -20°C for at least 30 minutes. Then centrifuged at maximum speed (≥12000 rpm) for 15 minutes at 4°C to collect the RNA pellet.
e. Wash the RNA pellet by adding 500 μL of ice-cold 70% ethanol and centrifuge at maximum speed (≥ 12,000 rpm) for 5 minutes at 4°C.
f. Dissolve the RNA pellet in 20 μL of Nuclease-Free Water. Store the purified RNA solution at -80℃.

## 2) Column purification

Column purification can remove proteins and free nucleotides. To begin, dilute the product by adding 80 μL of Nuclease-Free Water to make a total volume of 100 μ L, then, purify according to the column instructions.

***Note: Due to high RNA yield, ensure that the column capacity is sufficient to avoid exceeding its binding capacity***.

## 3) Magnetic bead purification

Follow the instructions for magnetic bead purification.

## 4) Lithium chloride precipitation

For lithium chloride precipitation, the RNA length should be at least 100 nt, and the RNA concentration should not be less than 100 ng/μL.

a. Add 30 μ L of Nuclease-Free Water and 30 μ L of 7.5 M lithium chloride to 20 μ L of the reaction mixture. Mix well.

b. Incubate at -20 °C for at least 30 minutes. Then, centrifuge at maximum speed (≥12000 rpm) for 15 minutes at 4°C, and collect the pellet.

c. Wash the RNA pellet by adding 500 μL of ice-cold 70% ethanol, then centrifuge at maximum speed (≥ 12,000 rpm) for 5 minutes at 4°C.

d. Dissolve the RNA pellet in 20 μL of Nuclease-Free Water. Store the purified RNA solution at -80℃.

## 5. RNA Quantification and Detection

RNA quantification

1) Quantification by UV Light Absorbance: Free NTP and other items can significantly affect the accuracy of quantification, please purify RNA before using this method.

2) Dye binding assays: RNA quantification can be performed using RNA-specific fluorescent dyes or related kits. Free nucleotides do not interfere with this method, allowing for the quantification of RNA in both purified and unpurified reaction products.

## RNA size and quality assays

1) **Gel Electrophoresis**

To assess transcript length, integrity and quantity, an aliquot of the transcription reaction should be loaded onto an appropriate denaturing agarose gel or polyacrylamide gel. Denaturing electrophoresis minimizes the formation of RNA secondary structures, allowing the RNA to migrate as a single band of the correct size.

Note: Transcription RNA can be diluted with Nuclease-Free Water and electrophoresis. The recommended electrophoresis load is 0.05-1 μ g. Samples can be treated at 65 ° C for 5 – 10 minutes after adding RNA Loading Buffer to reduce RNA secondary structure formation.

2) **Capillary electrophoresis**

Capillary electrophoresis provides a high accurate digital assessment of RNA sample integrity, purity, and degradation. Compared to the gel method, this method requires a small amount of RNA and offers higher sensitive.

# FAQs

1. Low yield of RNA transcript

The template is closely related to the yield. If the yield is normal in the control group but low in the experimental group, it could be due to the inhibitory components in the experimental template or inaccurate concentration due to the poor template purity. It is recommended to re-purify the template to confirm its concentration and integrity, or to extend the reaction time at 37 °C. Increasing the amount of template input can also help improve the yield.

2. Low yield of short transcript

Short transcription fragment can inhibit the reaction. When the transcript product is ＜ 100 nt, it is recommended to extend the reaction time or increase the template amount to 2 μg.

3. The size of RNA transcript is smaller than expected

① The template sequence may contain a termination sequence similar to that of T7 RNA polymerase. It is recommended to lower the reaction temperature to 30℃, which may increase the proportion of full-length transcripts, although the yield might decrease.

② High GC content in the template or the formation of secondary structures could be an issue. It is recommended to increase the reaction temperature to 42°C or use single-stranded binding protein (SSB) to improve transcription efficiency.

③ If the RNA transcript size is not as expected, use a denaturing gel for detection.

4. The size of RNA transcript is larger than expected

① The template plasmid DNA may not have been completely digested. It is recommended to continue the restriction enzyme digestion until the plasmid is fully linearized.

② The RNA may not be fully denatured, leading to secondary structure formation. Use a denaturing gel to detect the RNA transcript.

5. RNA transcript electrophoresis tailing

This may occur if the DNA template or handling has been contaminated with RNases, leading to RNA transcript degradation. The RNase inhibitors in the reagent system can only suppress trace amounts of RNase residues. To prevent this, re-purify the template DNA and dissolve it in water treated with Nuclease-Free Water or DEPC. Ensure that user wear masks and gloves during the experiment, change gloves frequently, use RNase-free consumables, and prepare all reagents with Nuclease-Free Water.

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