MB363_IFU_EN_V2401

NZY T7 High Yield RNA Synthesis kit

Catalogue number	Presentation
MB36301	50 reactions

Description

The NZY T7 High Yield RNA Synthesis kit is designed for the *in vitro* production of large quantities of RNA transcripts from any DNA template under the control of a T7 promoter sequence. This kit is formulated to yield high amounts of RNA transcripts, typically ranging from 150-200 µg of RNA from 1 µg DNA template in a 2-hour reaction as described below. The resulting RNA transcripts exhibit high integrity and meet the necessary quality standards for a variety of downstream applications, including RNA splicing studies or RNA-protein interaction experiments. The *in vitro* transcription reaction is catalysed by an engineered version of phage T7 RNA polymerase. This enzyme exhibits high specificity for the T7 promoter, facilitating efficient transcription of the DNA strand of interest from the promoter sequence. The NZY T7 High Yield RNA Synthesis kit is suitable for transcribing linearized plasmid DNA templates or DNA templates generated by PCR. To preserve the integrity of the resulting RNA transcripts, the kit components include a Ribonuclease Inhibitor. This protein effectively protects the synthesized RNA transcripts from degradation by contaminating RNases originating from various sources, such as plasmid templates or others.

The NZY T7 High Yield RNA Synthesis kit was optimized to produce large quantities of RNA transcripts using unmodified ribonucleotides. However, this kit is compatible with capped RNA synthesis or RNA synthesis using modified ribonucleotides.

Shipping & Storage Conditions

This product is shipped in dry ice. Upon receipt, store all components at -85 °C to -15 °C in a constant temperature freezer. Immediately after use, swiftly return all components to a temperature between -85 °C and -15 °C. Adhering to these meticulous storage procedures ensures that product delivers consistent and reliable results across its lifespan and usage. Stored as specified, the product will remain stable until the expiry date, ensuring reliable and consistent performance in all applications.

Components

	MB36301 (50 reactions)	
COMPONENT	**TUBES**	**VOLUME**
T7 HY Enzyme Mix	1	100 µL
10× HY Transcription Reaction Buffer	1	100 µL
100 mM ATP	1	50 µL
100 mM CTP	1	50 µL
100 mM GTP	1	50 µL
100 mM UTP	1	50 µL
Linear DNA Control (*)	1	10 µL

(*) Provided for 3 experiments. The control template DNA is a 6043 bp linearized plasmid which codes for a 600 nt RNA transcript.

Standard Protocol

Recommendations before starting

- Reagent usage: The T7 HY Enzyme Mix already includes a Ribonuclease inhibitor. Do not add extra RNase inhibitor. We highly recommend using sterile molecular biology-grade, nuclease-free water, preferably DEPC-treated water.
- Handling instructions:

- During setup, place the T7 HY Enzyme Mix and ribonucleotides solutions on ice but keep the 10× HY Transcription Reaction Buffer at room temperature. Some components present in the 10× HY Transcription Reaction Buffer can co-precipitate the template DNA if the reaction is assembled on ice.
- The reaction setup should be executed under stringent conditions to prevent contamination with RNases. This includes working in a dedicated RNase-free environment, using RNase-free reagents and consumables, and wearing gloves to minimize the introduction of RNases

from the skin. Additionally, it is crucial to regularly clean and decontaminate work surfaces and equipment. The RNase & DNase Cleaner (NZYtech, Cat. No. MB463) can help removing RNases from surfaces and materials.

Procedure for *in vitro* transcription

1. Gently mix all reaction components and pulse briefly
2. At room temperature, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture by combining the following components in the order indicated:
   Note: *If setting up more than one reaction, prepare a reaction mixture volume 10% greater than the total required for the number of reactions to be performed.*

COMPONENT	1 REACTION VOLUME
Nuclease-free water (not provided)	up to 20 µL
10× HY Transcription Reaction Buffer	2 µL
100 mM ATP	1 µL
100 mM CTP	1 µL
100 mM GTP	1 µL
100 mM UTP	1 µL
Template DNA (*)	0.5-1 µg
T7 HY Enzyme Mix	2 µL
FINAL VOLUME =	20 µL

(*) For the control reaction, use 3 µL of Linear DNA control provided (0.5 µg).

3. Mix thoroughly and centrifuge briefly to collect the contents at the bottom of the tube.
4. Incubate at 37 °C for 2 hours.
   Note: *For short transcripts (<0.3 Kb), incubate the transcription reaction at 37 °C for 4 hours or more.*
5. (**Optional**) To remove the DNA template, treat the reaction with DNase I. To achieve this, add 2 U DNase I (not provided, but ensure that it is RNase-free) directly to the transcription mixture and incubate at 37 °C for 15 minutes. Then, inactivate the DNase I by adding 2 µL EDTA at 0.2 M followed by heating at 70 °C for 10 minutes, or by phenol/chloroform extraction.
6. If not performing the DNase I treatment, inactivate the reaction by adding 2 µL EDTA at 0.2 M and/or heating at 70 °C for 10 minutes.
7. Store synthesized RNA at -85 °C to -65 °C or proceed to downstream applications.

Technical Notes

DNA Template Type

Any linearized plasmid or PCR products containing a T7 promoter region may be used as a template for high-yield *in vitro* transcription using the NZY T7 High Yield RNA Synthesis kit. The sequence of the T7 promoter recognized by T7 RNA polymerase (included into the T7 HY Enzyme Mix) is indicated in Figure 1:

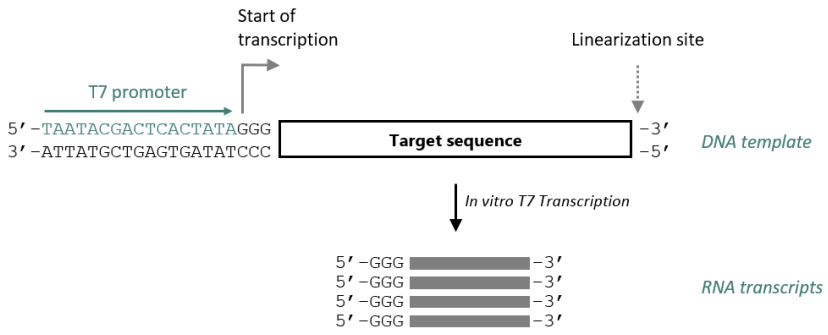

Figure 1. *In vitro* transcription from the T7 promoter.

The orientation of RNA synthesis (sense or antisense) depends on the orientation of the T7 promoter relative to the sequence of interest. Thus, for sense RNA transcripts, the sequence of interest must be placed downstream of the T7 promoter (see Figure 1).

Plasmid templates: The success of *in vitro* transcription using the NZY T7 High Yield RNA Synthesis kit depends on the quality and linearization of plasmid templates, as these parameters affect the quality and quantity of synthesized RNA transcripts. Plasmid templates must be linearized before the *in vitro* transcription reaction to enable the production of RNA transcripts with defined lengths. Circular plasmid templates are not recommended, as they will produce long and heterogeneous RNA transcripts due to the highly processive nature of T7 RNA polymerases. While the *in vitro* transcription reaction will cease when T7 RNA polymerase encounters a loop or hairpin termination region, its efficacy will vary depending on the type of terminator present. Therefore, when aiming to generate RNAs with defined lengths using plasmid templates, it is highly recommended to digest the DNA at the 3´-region where transcription is intended to stop. Thus, linearizing the plasmid template by restriction digesting the DNA at a position downstream of the insert to be transcribed is strongly advised (see Figure 1). Preferably, the DNA template should be double-digested upstream and downstream of the region to be transcribed. Restriction enzymes that generate blunt ends or 5' overhangs are preferred over those that produce 3' overhangs. If using the latter enzymes, the linearized plasmid ends can be made blunt using the Klenow Fragment of DNA polymerase I (NZYtech, Cat. No. MB009) prior to transcription.

PCR templates: DNA templates generated by PCR and containing a T7 promoter can be efficiently transcribed using the NZY T7 High Yield RNA Synthesis kit. Prior to their inclusion in the *in vitro* transcription reaction, PCR products should be assessed on an agarose gel to estimate their concentration and confirm the expected size. It is recommended to purify PCR products to achieve optimal transcription yields.

Amount of DNA Template

We recommend using 0.5-1 µg of any DNA template in a 20 µL transcription reaction.

Quality control

Purity

The T7 HY Enzyme Mix components are >90% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Nucleases assay

To test for DNase contamination, 0.2-0.3 µg of pNZY28 DNA are incubated with the kit component in test for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the kit component in test for 1 h at 37°C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids. Similar tests are performed with reaction buffer.

Functional assay

The kit is functionally tested in an *in vitro* transcription reaction. The result must be 150 µg of intact RNA transcript produced from 1 µg of linearized DNA template