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High-yield T7 RNA synthesis kit

Cat. Number: T7-tr20, T7-tr100

Description:

High-yield T7 RNA synthesis kit (T7-tr20, T7-tr100) is designed to produce RNA via *in vitro* transcription with T7 RNA polymerase.

Linearized plasmid DNA or PCR products containing a double-stranded G-initiating T7 promotor region upstream of the target sequence can be used as template for *in vitro* transcription with the High-yield T7 RNA synthesis kit (Figure 1).

The resulting RNA can subsequently be used for a variety of applications such as microinjection, transfection or *in vitro* translation experiments, structure/function studies or probes for *in situ* hybridization and northern blot experiments.

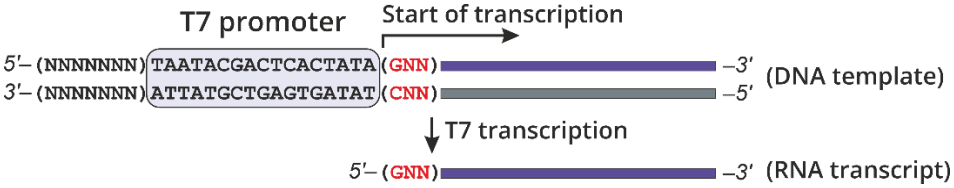

Figure 1. Transcription by T7 RNA polymerase.

Note! Minimum T7 promoter sequence:
5′–NNNNNNNTAATACGACTCACTATAGNN…–*3′*.
First base incorporated into RNA: **G**; next NN: ideally **CG**.

Note! High-yield T7 RNA synthesis kit is also suitable for the incorporation of modified nucleotides (e.g. pseudouridine, 5-methylcytidine; biotin- or fluorescent-labeled NTPs; cap analogs (ARCA)).

Kit contains:

Component	T7-tr20 (20 reactions)	T7-tr100 (100 reactions)
(×5) T7 reaction buffer	240 µl	1,2 ml
(×25) DTT	50 µl	250 µl
T7 RNA polymerase	25 µl	125 µl
NTP mix	45 µl	230 µl
Nuclease-free water	1 ml	4 ml

(×5) T7 reaction buffer
Tris-based buffer, salts, and other ingredients

(×25) DTT
250 mM DTT

Nuclease-free water

T7 RNA polymerase
buffered 50% (v/v) glycerol containing 150 e.a./µl T7 RNA polymerase

NTP mix
25 mM of each NTP
(ATP, CTP, GTP и UTP)

Equipment to be supplied by user

- Thermostat with the ability to keep the temperature at 37°C.
- 0.6 or 1.5 ml microcentrifuge tubes.
- Microcentrifuge.

> **Note!** Creating a RNAse-free work environment and maintaining RNAse-free solutions is critical for performing successful *in vitro* transcription reactions. We therefore recommend using RNase inhibitor.

Companion products

DNase (EM-100, Biolabmix).

RNase inhibitor (RI-0020, Biolabmix).

Pseudouridine-5'-triphosphate (TPU-0050, Biolabmix).

5-methylcytidine-5'-triphosphate (TMC-0050, Biolabmix).

Anti-reverse cap analog (ARCA) (ARCA-0050, Biolabmix).

RNA loading buffer (D-3001, Biolabmix).

RNA column isolation kit (RU-10, Biolabmix).

***In vitro* transcription protocol**

1. Reaction mix preparation
Place T7 RNA polymerase on ice. Thaw the necessary kit components, mix and pulse-spin in microfuge to collect solutions to bottom of tubes. Assemble the reaction at room temperature in the following order:

Component	Concentration	Final conc.	Volume
(×5) T7 reaction buffer	(×5)	(×1)	10 µl
(×25) DTT	(×25)	(×1)	2 µl
NTP mix	25 mM of each NTP	1 mM of each NTP	2 µl
DNA template	variable	variable	0.5–2 µg
T7 RNA polymerase	150 e.a./µl	3 e.a./µl	1 µl
Nuclease-free water			up to 50 µl
Total reaction volume			**50 µl**

2. Incubation
Mix thoroughly and pulse-spin in a microfuge. Incubate at 37°C for 2 hours.

3. DNase treatment to remove template DNA
Add 2 e.a. of DNase I, mix well and incubate at 37°C for 15 minutes. DNase treatment is optional if the template does not interfere with downstream experiment.

> **Note!** The protocol is optimized for 0.5–2 µg DNA template, 1 mM each NTP.

> **Note!** A 50 µl reaction yields about 10–30 µg RNA after 2 hours incubation (1 µg DNA template, 1.2 kb RNA transcript). Yields may vary depending on the template (promotor design, sequence length, secondary structure formation).

Individual optimization of protocol design
Linearized plasmid DNA or PCR products containing a double-stranded G-initiating T7 promotor region upstream of the target sequence can be used as template for *in vitro* transcription. Linearized plasmid DNA needs to be fully digested. We recommend to purify the DNA template for *in vitro* transcription.

Incubation time depends on template amount, quality and RNA transcript length. For reactions with transcripts longer than 0.5 kb, 2 hours incubation should give you the maximum yield. For reactions with short RNA transcripts (< 0.5 kb), incubation time of 4 hours or longer is necessary to achieve good yield. It is safe to incubate the reaction for 16 hours (overnight).

RNA analysis
RNA can be analyzed by gel electrophoresis in 1–2,5% agarose gel.

RNA can be purified by LiCl precipitation, phenol:chloroform extraction followed by ethanol precipitation, or by using a spin column based method.

The amount of the purified RNA can be estimated using UV spectrometry. The maximum of absorption for RNA corresponds to $\lambda = 260\ nm$. RNA concentration (µg/ml) can be calculated using the following formula: $A260 \times dilution \times 40\ \mu g/ml$. Typical optical density ratios are $A_{260}/A_{280} \geq 1.8\text{-}2.0$, $A_{260}/_{230} \geq 1.9$.

Storage
All kit components should be stored at −20 °C for up to 12 months.