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Product Information EzRNA™ series

T7 High Yield RNA Synthesis Kit (me$^{1}\Psi$-UTP)

IT1200 50 RXN

T7 RNA Polymerase Mix	100 µl
10X T7 Reaction Buffer	100 µl
NTP (me$^{1}\Psi$) Premix (25 mM each)	400 µl
Control DNA (0.5 µg/µl)	10 µl
Lithium Chloride (7.5M)	1 ml
Nuclease-Free Water	1 ml

Storage

-20°C for 12 months

Description

The EzRNA™ T7 High Yield RNA Synthesis Kit ($me^1\Psi$-UTP) is a user-friendly product for enzymatic RNA production. The enzyme mix contains adequate amount of T7 RNA polymerase, pyrophosphatase, and RNase inhibitors for *in vitro* transcription (IVT). Along with 10X Transcription Buffer and NTP ($me^1\Psi$) Premix, users can swiftly assemble IVT reactions without compromising RNA yield. The EzRNA™ T7 High Yield RNA Synthesis Kit ($me^1\Psi$-UTP) allows for the attainment of approximately up to 130 µg RNA yield within 2 hours at 37°C.

Features

- High yield
- Versatile- suitable for short and long transcripts
- NTP premixed- Minimal pipetting and setup time
- Compatible with CleanCap® Reagent AG
- Lithium chloride included for RNA purification

Application

- Generation of RNA from T7 promoter-driven DNA sequences.
- Suitable for subsequent cap-0 and cap-1 modification.

RNA Synthesis Condition

1. Recommended IVT reaction set-up:

Linearized DNA	1 µg
10X T7 Reaction Buffer	2 µl
NTP ($me^1\Psi$) Premix (25 mM each)	8 µl
T7 RNA Polymerase Mix	2 µl
Nuclease-Free Water	to 20 µl
Total volume	20 µl

<Note>

- Except T7 RNA polymerase mix, thaw all of other reaction components fully and assemble reaction at room temperature.
- Reaction can be scaled-up proportionally.
- To avoid nuclease contamination, ensure assemble reaction in a clean environment.

2. Recommended IVT condition:

Incubate reaction at 37°C for 2 hours. To achieve maximum yield, reactions could be optimized and extend reaction time to 4~16 hours based on amount of DNA template and/or the size of IVT transcript.

3. Control reaction:

For performance verification, use the provided Control DNA (linearized form).

- Expected RNA length: ~2,110 nt.
- RNA sequence features: Contains an encoded poly(A) tail and incorporates me$^{1}\Psi$-UTP modified nucleotides.

RNA product purification

There are several options of RNA purification listed below:

1. Lithium chloride (LiCl) purification

Lithium chloride could specifically precipitate RNA molecules without additional ethanol or isopropanol. Along with free nucleotides and proteins in IVT reaction, DNA template would be retained in supernatant when using LiCl. It's important to note that this method requires a certain amount of RNA (> 0.1 μg/μl) and a minimum length of RNA (> 300 bases typically) to be effective.

Brief procedure:

Add 1/2 volume of LiCl into RNA sample and mix thoroughly → -20°C incubation for at least 1 hour → 4°C, 14000× g centrifugation for at least 15 minutes → remove supernatant and ice-cold 70% ethanol wash at least twice → air-dry pellet shortly and dissolve RNA pellet with water or buffer for certain time period (depend on pellet size).

2. Phenol-chloroform extraction and ethanol precipitation

By virtue of phase separation, protein and free nucleotides would be brought into organic layer completely. Nucleic acid (RNA and DNA) in water phase could be further purified by ethanol precipitation. Acidic phenol could differentially retain RNA molecules but not DNA in water phase.

3. Spin column purification

Certain buffer compositions enable the specific purification of RNA using silica-based columns. Moreover, oligo-dT column are able to purify poly(A)-containing RNA e.g. mRNA. It is important to adhere to the instructions provided in the column purification kit for proper usage.

RNA product assessment

- **Gel electrophoresis**

Compared to DNA, RNA (especially long RNA) molecules may have numerous of secondary structures. For rigorous evaluation, it is essential to generate denatured condition for RNA gel electrophoresis. This typically involves using denatured RNA samples (RNA samples with denatured loading dye) in either denatured agarose gel or polyacrylamide gel. Due to the properties of different gel mobility, it is necessary to use an RNA size ladder rather than the regular DNA ladder for accurate RNA size determination.

- **RNA quantification**

Standard approach is UV absorbance at 260 nm (A260). Concentration of RNA can be calculated as A260 x 40 µg/ml. Note that the presence of any impurity in the samples (such as protein, free nucleotides, or DNA) would affect the absorbance reading, leading to an overestimation of RNA quantity.

Other Information

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Caution: Not intended for human or animal diagnostic or therapeutic uses.

Related Products

RC1000	EzRNA RNA Capping System, 50 RXN
IT1000	T7 High Yield RNA Synthesis Kit, 50 RXN
IT1100	EzRNA T7 High Yield RNA Synthesis Kit (Ψ-UTP), 50 RXN
RI1000	RNAok RNase Inhibitor, 2000 U
RP1400	ExcelRT Reverse Transcription Kit II, 100 RXN
TF1000	SMO-HiFi DNA Polymerase, 100 U
TF3000	G-HiFi DNA Polymerase, 100 U
TP5000	ExcelTaq Hot Start II DNA Polymerase, 500 U
TQ1110	ExcelTaq 2X Q-PCR Master Mix (SYBR, ROX), 200 RXN
TQ2110	ExcelTaq 2X Q-PCR Master Mix (TaqMan, ROX), 200 RXN
DM2100	ExcelBand 100 bp DNA Ladder, 500 µl
NS1000	FluoroVue Nucleic Acid Gel Stain (10,000X), 500 µl

The latest version of the manual can be downloaded from www.smobio.com/shop.

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2026 ver. 1.2.0