LEISH BIO

VENI T7 RNA Synthesis Kit

For *in vitro* use only!

Storage Conditions: store at -20 °C
Avoid freeze/thaw cycles

Shelf Life: 24 months after the date of delivery

Description:
This RNA Synthesis Kit is designed to produce large amounts RNA via *in vitro* transcription with T7 RNA Polymerase. The resulting RNA can subsequently be used for various applications such as microinjection, transfection, as gRNA to forms RNP with Cas9 protein or *in vitro* translation experiments.
The kit contains sufficient reagents for 20 reactions of 50 µl each. A typical reaction yields about 40-160 µg RNA after 2hrs incubation (1 µg T7 control template, 1.4 kb RNA transcript). Yields may vary depending on the template (promotor design, sequence length, secondary structure formation).

Content:

Item	**Quantity**
T7 RNA Polymerase Mix incl. RNase inhibitor, Pyrophosphatase and 50 % glycerol (v/v)	80 µl
T7 Reaction Buffer (10x)	100 µl
NTP (GTP, ATP, CTP, UTP) 100 mM	100 ul each
Precipitation solution	2 ml
DTT (100 mM)	50 ul
RNase-free H2O	1 ml

To be provided by user:
T7 Promotor-containing DNA template
RNA purification column
RNase-free DNase I

Important Notes:

Prevention of RNase contamination
Although a potent RNase Inhibitor is included, creating a RNase-free work environment and maintaining RNase-free solutions is critical for performing successful *in vitro* transcription reactions. We, therefore, recommend

- to perform all reactions in sterile, RNase-free tubes using sterile pipette tips.
- to wear gloves when handling samples containing RNA.

- to keep all components tightly sealed both during storage and reaction procedure.

Template requirements

Minimum T7 promotor sequences: 5′ -TAATACGACTCACTATAGGG

Template quality: DNA template quality directly influences yield and quality of transcription reaction. Linearized plasmid DNA needs to be fully digested and to be free of contaminating RNase, protein and salts. We recommend selecting restriction enzymes that generate blunt ends or 5´-overhangs and purification by phenol/chloroform extraction. A PCR mixture can be used directly however, better yields will usually be obtained with purified PCR products (e.g. via silica-membrane based purification columns).

- mRNA production: For the production of functional mRNA please ensure that the DNA template encodes the required structural features e.g. 3'-UTR, 5'-UTR, correctly orientated target sequence and poly A-tail. Alternatively, poly A-tailing can post-transcriptionally be performed with Poly A polymerase.

***In vitro* Transcription protocol:**

- Place T7 RNA Polymerase Mix on ice.
- Thaw all remaining components at room temperature (RT), mix by vertexing and spin down briefly.
- Assemble all components at RT to a nuclease-free microtube (sterile pipette tips) in the following order:
- Mix PCR-grade water, T7 Reaction Buffer and DTT by vertexing and spin down briefly.
- Add NTP solutions and template DNA, vortex and spin down briefly.
- Add T7 RNA Polymerase Mix vortex and spin down briefly.
- Incubate for 2h at 37°C in the dark (e.g. PCR cycler). Depending on the RNA sequence individual optimization may increase product yield (0.5h–4h at 37 °C).

Component	**Volume**	**Final concentration**
RNase-free H2O	-	
T7 Reaction Buffer (10x)	2 µl	1x
DTT (100 mM)	1 µl	10 mM
ATP 100mM	1.5 µl	7.5 mM
UTP 100mM	1.5 µl	7.5 mM
CTP 100mM	1.5 µl	7.5 mM
GTP 100mM	1.5 µl	7.5 mM
Template DNA	X µl	25-50 ng/µl
T7 RNA Polymerase Mix	1.6 µl	
Total volume	20 µl	

Note: for short RNA transcripts, it is recommended to use a higher concentration of template DNA to achieve optimal results.

DNA template removal:

Please note: Reagents for this step are not provided within this kit.
Depending on the downstream application, the removal of template DNA might be required. We recommend a salt-resistant, high-efficiency DNase.

RNA purification:
Add 25 µl (1/2 volume) Precipitation Solution, mix it and incubate for at least 15 min at -20 °C to facilitate the precipitation. Spin it down at 12000g for 15min. A white pellet should be visible. Wash the pellets with cold 70% alcohol and spin it down to remove the alcohol.

Dissolve the RNA pellet with 40 µl RNase-free water. A concentration of 2 µg/ul to 3 µg/ul will be expected, which may vary depending on RNA sequence.