LEISH BIO

VENI all-in-one mRNA Synthesis Kit with Cap1 Analog

For *in vitro* use only!

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

Shelf Life: 24 months after date of delivery

Description:
This all-in-one RNA Synthesis Kit with directly cap1 analog is designed to produce large amounts of cap1 capped N1-methyl-pseudoUTP modified RNA via *in vitro* transcription with T7 RNA Polymerase. The resulting RNA can subsequently be used for a variety of applications such as microinjection, transfection 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 however 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, N1-methyl-pseudo-UTP) 25 mM	300 ul
Cap 1 (100 mM)	20 µl
STOP solution 1 ml	1 ml
Precipitation solution 2 ml	2 ml
DTT (100 mM)	50 ul

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

Important Notes (Read before starting)

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′ -TAATACGACTCACTATAAGG

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 nucleotide 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 conc.**
H2O	-	
T7 Reaction Buffer (10x)	5 µl	1x
DTT (100 mM)	2 µl	10 mM
NTP (GTP, ATP, CTP, N1-methyl-pseudo-UTP) 25mM	15 µl	5 mM
Cap1 (100 mM)	2 µl	4 mM
Template DNA	X µl	50 ng-100n g/ul
T7 RNA Polymerase Mix(5 U/ul)	4 µl	
Total volume	50 µl	

DNA template removal

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

RNA purification
Add LiCl precipitation buffer, mix it and incubate for 15min at -20 °C to facilitate the precipitation. Spin it down at 12000g 15min. Wash the pellets with cold 70% alcohol and spin it down to remove the alcohol.

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

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