LEISH BIO

VENI all-in-one mRNA Synthesis Kit with Enzymatic Capping

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 all-in-one RNA Synthesis Kit is designed to produce large amounts of cap0 capped N1-methyl-pseudoUTP RNA via *in vitro* transcription with T7 RNA Polymerase. The resulting RNA is optimized for high protein production. The kit contains sufficient reagents for 20 reactions of 50 µl each. A typical reaction yields about 40-160 µg RNA after 2hr 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).

Contents:

Item	Quantity
T7 RNA Polymerase Mix	80 µl
T7 Reaction Buffer (10x)	100 µl
NTP (GTP, ATP, CTP, N1-methyl-pseudo-UTP) 25mM	300 ul
GTP (100 mM)	20 µl
Precipitation Solution	2 ml
RNA Capping Enzyme Mix	50 ul
RNA Capping Buffer (10x)	100 ul
SAM (32 mM)	10 ul
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 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 optimization may increase product yield (0.5h–4h at 37 °C).

Component	**Volume**	**Final concentration**
RNase-free H2O	-	
T7 Reaction Buffer (10x)	5 µl	1 x
DTT (100 mM)	2 µl	4 mM
NTP (GTP, ATP, CTP, N1-methyl-pseudo-UTP) 25mM	15µl	7.5 mM
Template DNA	X µl	25-50 ng/µl
T7 RNA Polymerase Mix(5U/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 25 µl (1/2 volume) Precipitation Solution, mix it and incubate for 15 min 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 40 μl RNase-free water. A concentration of 2 μg/μl to 3 μg/μl will be expected, which may vary depending on sequence of the RNA.

RNA Capping:

Component	**Volume**
RNA Capping Buffer (10x)	5 µl
GTP (100 mM)	0.5 µl
SAM (32 mM)	0.5 µl
mRNA IVT production(2 ug/ul-3 ug/ul)	41.5 µl
RNA Capping Enzyme Mix	2.5 µl
Total volume	50 µl

Incubate at 37 °C for 1 hour. And ½ volume of Precipitation Solution and incubate at -20 °C at least 1 hour. Spin it down at 12000 g for 15 min at 4°C to precipitate the RNA. Wash with cold 70% alcohol and spin it down to remove the alcohol. Dissolve the RNA pellet with 40μl RNase-free water.