AMCAP™ INTERMEDIATE IVT KIT (Pseudo-Uridine modified)

Ready-to-go IVT Kit using AMCAP™ Enzymatic capping Technology

Catalog # KAMC.003

1. Description

ANEMOCYTE AMCAP™ Intermediate mRNA IVT Kit represents the all-in-one solution for generating high-quality, Cap-1 messenger RNA.

Key Features and Benefits:

- Innovative One-Pot Synthesis: The kit utilizes the patent-pending AMCAP™ process, combining In Vitro Transcription (IVT) and Capping in a single, streamlined reaction (ONE POT synthesis).
- Superior Capping Efficiency: Achieve significantly higher levels of the critical Cap-1 structure on the 5' end of your mRNA compared to standard two-step enzymatic capping. The AMCAP™ technology is shown to deliver high mRNA capping efficiency.
- High Yield: The AMCAP™ reaction delivers high mRNA production yield compared to both standard two-step enzymatic capping and co-transcriptional processes.
- Enhanced Protein Expression: mRNA synthesized with AMCAP™ shows a higher level of protein expression in transfected cells compared to standard two-step enzymatic capping.
- Reduced Processing Steps and Time: By eliminating separate capping steps, the AMCAP™ technology reduces overall processing time, costs, and the number of required steps, all while maintaining high mRNA quality.

AMCAP™ Intermediate mRNA IVT Kit is ready to use and is available with Pseudo-Uridine (Ψ) modification.

2. Usage and Storage

2.1. Materials Supplied

Each AMCAP™ Intermediate mRNA IVT Kit contains reagents for 5 x 100 µL or 25 x 20 µL reactions.

Store at -15 °C to -20 °C. Avoid repeated freeze-thaw cycle, aliquot samples. Use only certified RNase-free reagents and consumables with proper RNase-free technique.

Materials provided with the Kit:

- 1 vial of AMCAP™ IVT Intermediate Mix containing N1-Methyl Pseudouridine (Ψ) (0.406 mL)
- 1 vial of pmRNA_F-Luc linearized template pDNA (0.03 mL)[1]

[1] Linearized Control Template DNA: is a linearized plasmid that contains a T7 promoter followed by a F-Luc insert and a polyA tail. The Control Template DNA is provided at a concentration of 1 µg/µL.

2.2. Materials Required but not Supplied

- A DNA template for transcription of your RNA of interest containing a T7 promoter and a polyA tail
- RNase-free Water
- Materials or kits for purification of the RNA product
- RNase-free 1 mM sodium citrate buffer pH 6.4
- RNase-free laboratory plasticware and standard laboratory equipment

2.3. Optional Reagents (Not Supplied)

- DNase I (RNase-free)
- RNase Decontamination Solution

2.4. Functional testing

Each kit is tested to meet our standard QC specifications. Each kit lot is functionally verified to yield a F-Luc mRNA following our recommended protocol with the included control.

2.5. Essential Pre-Experiment Tips for optimal In-Vitro transcription

1. Pre-requisites for In Vitro Transcription Template Synthesis

The successful execution of in vitro transcription (IVT) and its downstream applications necessitates that the user assumes responsibility for the design and procurement of the requisite template sequences.

The template must incorporate the mandated T7 promoter sequence (5'TAATACGACTCACTATA 3'). Compatibility is confirmed for the AMCAP™ Intermediate mRNA IVT Kit with the 5' AG 3' or 5' GG 3' initiating sequences. Furthermore, generation of a polyadenylated mRNA transcript requires the polyA tail to be template-encoded.

The preferred templates are linear dsDNA molecules, which can be obtained through one of two methods:

- Enzymatic Linearization: Digesting a circular dsDNA vector (such as a plasmid) containing the cloned sequence with an appropriate restriction endonuclease at a site located immediately 3' to the polyA tail.

- PCR Generation: Employing Polymerase Chain Reaction to amplify the sequence of interest, utilizing a primer strategy that successfully integrates the T7 promoter at the designated end of the resulting amplicon.

2. Template Efficiency and Incubation Time:

In vitro transcription of 5 µg of provided Control Template DNA using the AMCAP™ Intermediate mRNA IVT Kit yields approximately 300-500 µg mRNA in a standard 100 µl reaction.

However, yields vary for different templates based on the template sequence, structure, length, or purity.

3. Reaction optimization

The recommended reaction conditions should give excellent yields of RNA with most templates.

However, the yield may be improved for some templates by extending the reaction time or increasing the amount of template in the reaction.

4. Maintaining an RNase-Free Environment

Since highly stable RNases are persistent (even on human skin), meticulous lab practices are critical for RNA integrity. Maintaining a strictly RNase-free environment and solutions is key to success.

We recommend the following essential steps:

- Ensure the exclusive use of RNase-free tubes and tips.
- Handle all kit reagents and RNA samples exclusively with gloves. Develop a habit of frequently changing gloves, particularly after contact with surfaces prone to RNase accumulation (e.g., equipment, writing tools, and your own skin).
- Keep all reaction and storage tubes tightly closed when not being actively used, including during all incubation periods.

2.6. Procedure

- Thaw the vial containing the AMCAP™ IVT Intermediate Mix ready to use. Vortex thoroughly for 30 sec. Mix stored at –20 °C may result in the formation of a white precipitate. To dissolve it, vortex for 1 minute.
- Set up the transcription reaction at room temperature following the scheme reported in the Table 1 below:

Component	Final concentration	Amount per 20 µL rxn	Amount per 100 µL rxn
AMCAP™ IVT Intermediate Mix[2]	N/A	13,5 µL	67,6 µL
DNA template [3]	50 ng/µL	1 µg	5 µg
RNase-free Water[4]	N/A	Up to 20 µL	Up to 100 µL

Table 1: Reaction setup

[2] AMCAP™ IVT Intermediate Mix ready to use already contains RNase inhibitor and inorganic pyrophosphatase.

[3] When using pmRNA_F-Luc linearized pDNA control template, add 1 µL of the linearized plasmid in a 20 µL of IVT reaction or 5 µL of the linearized plasmid in a 100 µL of IVT reaction.

[4] Calculate µL of water to add by subtracting the AMCAP™ IVT Intermediate Mix and DNA template volume to the final volume of reaction.

- If the concentration of linearized DNA is higher, dilute it at 1 µg/µL using Nuclease-free water.
- (Optional) Prepare one control reaction using the provided pmRNA_F-Luc linearized pDNA template.
- Vortex thoroughly for 30 sec.
- Incubate the reaction at +37 °C for three – four hours. To prevent evaporation and condensation on the tube lid, use a thermal cycler with a heated lid or a dry air incubator.
- (Optional) Treat reaction mixture with DNase I to remove the DNA template. Use 5 µL of RNase-Free DNase I concentrated 1U/µl for 100 µL IVT reaction.
- Proceed to RNA purification by traditional methods such as lithium chloride precipitation or spin columns for higher purity at small scales. Note: recovery of your mRNA from the purification process may vary.

3. Troubleshooting

Trouble	Possible Solution
Low yields or less than full- length transcripts	Cleanup the templates to remove any RNase or other contaminants
	Extend the incubation time. Do not extend the reaction time beyond 4 hours
	Increase the template concentration
	Check for stable secondary structure in the DNA template which can cause T7 RNAP pausing or dissociation from the template.
Assembled Reaction forms precipitate	Repeat assembly of the reaction at >22 °C
Precipitate in the AMCAP™ IVT Intermediate mix	Mix thoroughly to dissolve the precipitate.
	Do not store the kit at –70°C

4. Intended Use

For Research Use Only. Not for use in humans. Not for use in diagnostic or therapeutic purposes. The purchaser has sole responsibility for all and any use of the products.

5. Data & IP Statements

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