

## ScriptCap™ m<sup>7</sup>G Capping System

Cat. Nos. SCCE0610 and SCCE0625

The ScriptCap™ m<sup>7</sup>G Capping System, with Vaccinia Virus Capping Enzyme (VCE), provides a convenient, highly efficient, single-enzyme capping system to quantitatively build cap 0 structures on the 5' end of any amount of RNA *in vitro*.<sup>1</sup> VCE contains all three enzymatic activities (mRNA triphosphatase, guanylyltransferase and guanine-7-methyltransferase) necessary to catalyze the construction of N7-monomethylated cap 0 structures (m<sup>7</sup>G[5']ppp[5']NpN...).<sup>2-5</sup> Caps play a crucial role in maintaining the stability and translational efficiency of the mRNA *in vivo*.

With the ScriptCap m<sup>7</sup>G Capping System, capping efficiencies approach 100%, reactions can be scaled up or down to accommodate the user's capped RNA yield needs and the cap structures are all built in the proper orientation. In contrast, the traditional *in vitro* cap analog-based capping method of co-transcriptional cap 0-capped RNA production<sup>6</sup> has several drawbacks. Namely: because capping efficiencies are limited by the cap analog to GTP ratio, they can never be 100% and are often lower than the theoretical maximum;<sup>7,8</sup> reaction yields are greatly reduced (≤33%) due to the necessary limiting amount of GTP in the reaction; and some cap analogs can be incorporated in a reverse (backward) orientation further reducing the amount of properly-capped RNA produced in the reaction.<sup>9,10</sup>

The ScriptCap m<sup>7</sup>G Capping System can be used in conjunction with the ScriptCap 2'-O-Methyltransferase to produce cap 1-capped RNA (m<sup>7</sup>G[5']ppp[5']<sup>[m<sup>2</sup>-O]</sup>NpN...) which can further increase the translation efficiency of the mRNA *in vivo*.<sup>11,12</sup> ScriptCap m<sup>7</sup>G Capping System reactions can be added directly to Poly(A) Polymerase reactions for convenient 3'-end poly(A)-tailing of the capped RNA.

### ScriptCap™ m<sup>7</sup>G Capping System Contents

The ScriptCap™ m<sup>7</sup>G Capping System is available in both 10- and 25-reaction sizes (60 µg of RNA per reaction). The 25-reaction size kit contains:

ScriptCap™ Capping Enzyme @ 10 U/µl.....	100 µl
provided in red capped tube	
10X ScriptCap™ Capping Buffer.....	250 µl
10 mM GTP Solution.....	250 µl
20 mM SAM Solution.....	30 µl
ScriptGuard™ RNase Inhibitor @ 40 U/µl.....	65 µl
RNase-Free Water.....	1.8 ml

### Product Specifications

**Storage:** Store only at -20°C in a freezer without a defrost cycle.

**Storage Buffer:** ScriptCap Capping Enzyme is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM dithiothreitol, 0.1 mM EDTA, and 0.1% Triton® X-100.

**Unit Definition:** One unit of ScriptCap Capping Enzyme releases 1 nmole of inorganic phosphate from GTP in 10 minutes at 37°C under standard assay conditions.

**10X ScriptCap Capping Buffer:** 0.5 M Tris-HCl (pH 8.0), 60 mM KCl, and 12.5 mM MgCl<sub>2</sub>.

**Quality Control:** The ScriptCap m<sup>7</sup>G Capping System is function-tested for mRNA triphosphatase, guanylyltransferase, and guanine-7-methyltransferase activities in multiple assays.

**Contaminating Activity Assays:** All components of the ScriptCap m<sup>7</sup>G Capping System are free of detectable exo- and endonuclease and RNase activities.

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## Notes on Capping with the ScriptCap m<sup>7</sup>G Capping System

1. **SAM:** The SAM stock solution provided in the ScriptCap m<sup>7</sup>G Capping System is at a concentration of 20 mM. The final “working” capping reaction SAM concentration is 0.1 mM. Prior to use, we recommend that the user make up a fresh dilution of the 20 mM SAM stock in RNase-Free Water to a convenient concentration (e.g. 2 mM would be a 20X stock solution). The volume to make up would depend on how much SAM is needed for the intended reactions. The diluted SAM should be stored at –20°C, and can be used for up to one month post dilution.
2. **SAM:** SAM slowly degrades over time at room temperature and above. Keep thawed SAM solutions on ice.
3. **RNA Source:** RNA produced in an *in vitro* transcription reaction should be purified prior to addition to a ScriptCap m<sup>7</sup>G Capping System reaction. The final resuspension of the RNA should be in a non EDTA-containing solution, preferably RNase-Free water.
4. **10X ScriptCap Capping Buffer:** Due to storage conditions, a white precipitate may form in the 10X ScriptCap Capping Buffer stock tube. If this happens, heat the tube to 37°C for 5 minutes and mix thoroughly to resuspend the precipitate.
5. **RNA Secondary Structure:** Some RNA transcripts have the ability to form stable secondary structures (homodimers and hairpins) which involve the 5'-most nucleotides of the transcript. These RNAs require longer heat denaturation and capping reaction times (as indicated in the protocols on page 3) in order to increase the capping efficiency.
6. **Modification to Cap 1 Caps:** The ScriptCap m<sup>7</sup>G Capping System produces cap 0-capped RNA. If cap 1-capped RNA is desired, ScriptCap 2'-O-Methyltransferase (sold separately) can be added directly to the ScriptCap m<sup>7</sup>G Capping System reaction either simultaneously or sequentially without prior reaction clean-up.
7. **Poly(A)-Tails:** If the capped transcripts require subsequent poly(A)-tailing, it is not necessary to purify the capped RNA prior to poly(A)-tailing. When using EPICENTRE's Poly(A) Polymerase (sold separately), simply add 10X Poly(A) Polymerase Reaction Buffer (to a final concentration of 1X), ATP (to a final concentration of 1 mM) and the appropriate amount of Poly(A) Polymerase to the completed capping reaction and incubate at 37°C for the appropriate amount of time depending on the length of poly(A)-tail desired (see product literature). Capped and tailed RNA must be purified prior to use in RNA transfection experiments.
8. **Reaction Scale-up:** Reactions scaled-up in proportions different than those of a “Standard” reaction (e.g. 60 µg RNA in 100 µl total volume for 30 minutes) may require longer incubation times.

## Protocols

**Standard Cap 0 Capping Protocol** (designed for use on 50-60 µg of RNA)**A. Heat Denature the RNA**

1. In one tube, combine the following reaction components:

x	µl	RNase-Free Water
x	µl	<i>In vitro</i> transcribed RNA (50-60 µg RNA; <b>see Note 3 on page 2</b> )
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68.5	µl	Total reaction volume

2. Incubate at 65°C for 5-10 minutes (**see Note 5 on page 2**).
3. Transfer the tube immediately to ice.

**B. Cap the RNA**

4. *On ice*, combine the following reaction components in the order given:

68.5	µl	Heat denatured RNA from step A (above)
10	µl	10X ScriptCap Capping Buffer
10	µl	10 mM GTP
5	µl	2 mM SAM (add SAM to achieve a final concentration of 0.1 mM; <b>see Note 1 on page 2</b> )
2.5	µl	ScriptGuard RNase Inhibitor (40 U/µl) ( <b>Optional</b> )
4	µl	ScriptCap Capping Enzyme (10 U/µl)
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100	µl	Total reaction volume

5. Incubate at 37°C for 30-60 minutes (**see Note 5 on page 2**).

**Alternate Cap 0 Capping Protocol** (designed for use on 1-10 µg of RNA)**A. Heat Denature the RNA**

1. In one tube, combine the following reaction components:

x	µl	RNase-Free Water
x	µl	<i>In vitro</i> transcribed RNA (1-10 µg RNA; <b>see Note 3 on page 2</b> )
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13.5	µl	Total reaction volume

2. Incubate at 65°C for 5-10 minutes (**see Note 5 on page 2**).
3. Transfer the tube immediately to ice.

**B. Cap the RNA**

4. *On ice*, combine the following reaction components in the order given:

13.5	µl	Heat denatured RNA from step A (above)
2	µl	10X ScriptCap Capping Buffer
2	µl	10 mM GTP
1	µl	2 mM SAM (add SAM to achieve a final concentration of 0.1 mM; <b>see Note 1 on page 2</b> )
0.5	µl	ScriptGuard RNase Inhibitor (40 U/µl) ( <b>Optional</b> )
1	µl	ScriptCap Capping Enzyme (10 U/µl)
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20	µl	Total reaction volume

5. Incubate at 37°C for 30-60 minutes (**see Note 5 on page 2**).

**References:**

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**Related Products:** The following products are also available:

- AmpliScribe™ T7-Flash™ Transcription Kits
- AmpliScribe™ High Yield Transcription Kits
- Poly(A) Polymerase Tailing Kit
- ScriptCap™ 2'-O-Methyltransferase
- mScript™ mRNA Production System
- ScriptGuard™ RNase Inhibitor

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