

Prima RNAPols[®] Cap AG

DESCRIPTION

Prima RNAPols[®] Cap AG supports >97% AG capping with reduced cap analog input, ultra-low dsRNA (>100x reduction), robust yield and integrity across template and UTR designs, and highly translatable RNA with strong protein expression.

USE & HANDLING Store at -20 °C.

THE KIT CONTAINS

COMPONENT	VOLUME	PART NUMBER	CONCENTRATION
Prima Cap AG	100 µL	PBREC1-0.1	200 U/µL
2 kb Linearized Luc DNA Template	25 µL	PBT-0.025	250 ng/µL
5x IVT Reaction Buffer	400 µL	PBB2-0.1	5x

DNA TEMPLATE COMPATIBILITY

Prima Cap AG is only compatible with the optimized Prima Cap AG promoter. This sequence must be incorporated into the DNA template of interest to successfully generate RNA during in vitro transcription reactions. Prima Cap AG does not work on templates with the T7 promoter.



Figure 1. Required primer design to utilize Prima Cap AG. The TSS (AG) follows the promoter and is not part of the promoter sequence.

INCORPORATION OF PRIMA CAP AG PROMOTER SEQUENCE

The Prima Cap AG promoter sequence can be incorporated into a DNA template by either PCR or through cloning into plasmid DNA.

PCR-GENERATED TEMPLATES

1. For PCR-generated DNA templates, incorporate the Prima Cap AG promoter sequence directly upstream of the transcript to be generated. We recommend designing a forward primer that includes the promoter sequence and transcription start site (TSS) plus an additional 20 bases of homology to your UTR sequence (Figure 1).
2. Perform a DNA cleanup step and verify the construct for accuracy before running an IVT reaction.

RNASE-FREE

NOTE: All reagents must be RNase-free. Use disposable RNase-free tubes and bottles. When possible, use dedicated RNase-free pipettes. Avoid using pipettes that have been used for plasmid preparation using RNase A. Reactions should be assembled in a nuclease-free reaction vessel.

LINEARIZED PLASMID DNA

1. Incorporate the promoter and TSS into a plasmid directly upstream of the transcript to be generated using seamless molecular cloning techniques.
2. The plasmid DNA should be linearized by restriction enzyme digestion downstream of the transcript encoding region.
3. Perform a DNA cleanup step and verify the construct for accuracy before running an IVT reaction.

REAGENTS NOT INCLUDED IN THE KIT

- Nucleoside-5'-Triphosphate (NTP) Set (Thermo Scientific NTP Set (100 mM), Part # R1481, or equivalent)
- AG Cap Analog
- Inorganic pyrophosphatase (Recommended) (Thermo Scientific, Part # EF0221)
- RNase inhibitor (Recommended) (Thermo Scientific, Part # E00382, or equivalent)

PRIMA CAP AG IVT REACTION INSTRUCTIONS

PROCEDURE

1. Thaw 5x IVT buffer, NTPs, DNA template and cap analog at room temperature. Mix and pulse-spin to collect liquid to the bottom of the tubes. Keep at room temperature while in use.
2. Place Prima Cap AG, RNase inhibitor (recommended), and inorganic pyrophosphatase (recommended) on ice. Before use, mix and pulse-spin to collect liquid to the bottom of the tubes.
3. Assemble the IVT reaction in a microfuge tube at room temperature in the order shown in Table 1. The mixture can be scaled depending upon the number of IVT reactions and the volume of the reaction required.
4. Upon addition of the DNA template to the IVT reaction, mix and pulse-spin to collect liquid to the bottom of the tube. Add Prima Cap AG as the final component to the IVT reaction mixture. Mix thoroughly and pulse-spin. For optimal results, incubate at 37 °C for 3 hours with 350 rpm agitation.

TABLE 1. SUGGESTED IVT REACTION CONDITIONS FOR CAP AG

REAGENT	AMOUNT FOR 200 µL IVT	FINAL CONCENTRATION IN IVT REACTION
Nuclease-free water	Add water up to a final IVT volume of 200 µL	—
5x IVT Reaction Buffer	40 µL	1x
ATP (100 mM)	18 µL	9 mM
GTP (100 mM)	18 µL	9 mM
UTP (100 mM)	18 µL	9 mM
CTP (100 mM)	18 µL	9 mM
Cap analog (100 mM)	4 µL	2 mM
RNase inhibitor (40 U/µL) ¹	5 µL	1 U/µL
Inorganic pyrophosphatase (0.1 U/µL) ²	4 µL	0.002 U/µL
Template DNA ³	22 µL (5500 ng) ⁴	8 nM
MIX THOROUGHLY		
Prima Cap AG	20 µL	20 U/µL
Total reaction volume	200 µL	—

- 1 Addition of RNase inhibitor to the reaction is highly recommended.
- 2 Addition of inorganic pyrophosphatase to the reaction is highly recommended.
- 3 The 2 kb linearized DNA template provided has a 100 nt-long polyA tail and luciferase coding sequence.
- 4 Volume to be added to the IVT is based upon the 2 kb linearized DNA template (Part # PBT-0.025) provided at 250 ng/µL. This value may change depending upon the concentration of the DNA template used.