Prima RNApols™ ExTend Cap AU Kit

primrose bio™

Use & Handling
Store at -20°C.

The Kit Contains:

Kit Component	Part Number	Concentration
ExTend Cap AU RNA Polymerase (100 µL)	PBREC-0.1	200 U/µL
9.5 kb Linearized DNA Template (25 µL)	PBST-0.025	250 ng/µL
5x IVT Reaction Buffer (400 µL)	PBB1-0.1	5x

Description
Prima RNApols™ExTend Cap AU enables high-yield, fully capped self-amplifying mRNA using less reagents in every in vitro transcription (IVT) reaction. Built specifically for AU cap analogs, dsRNA reduction combined with lower reagent needs delivers higher quality, more cost-effective mRNA.

DNA Template Compatibility

ExTend Cap AU polymerase is only compatible with the ExTend Cap AU promoter. This sequence must be incorporated into the DNA template of interest to successfully generate mRNA during IVT. Prima ExTend Cap AU does not recognize the T7 promoter sequence.

Incorporation of ExTend Cap AU promoter sequence:
The ExTend Cap AU promoter sequence can be incorporated into a DNA template by either PCR or through incorporation into plasmid DNA.

PCR generated templates:

- For PCR generated DNA templates, we suggest incorporating the ExTend Cap AU 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).
- Perform a DNA cleanup step and verify the construct for accuracy before running an IVT reaction.

Linearized Plasmid DNA:

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

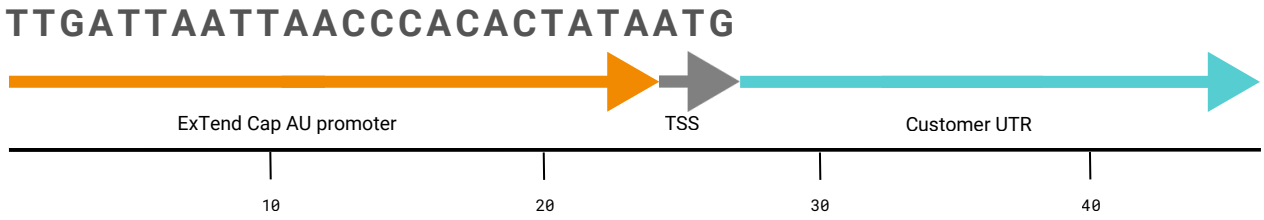

Figure 1: Recommended primer design to incorporate ExTend Cap AU Promoter sequence

Prima ExTend Cap AU IVT Reaction Instructions

Required Reagents
NOTE: All reagents must be RNase-free. Use recommended source or equivalent grade.

Reagents included in the Cap AU kit:

9.5 kb Linearized DNA template (Part # PBST-0.025).

ExTend Cap AU RNA polymerase (Part # PBREC-0.1)

5x IVT Reaction Buffer (Part # PBB1-0.1)

Reagents not included in the kit:

Nucleoside-5'-Triphosphate (NTP) Set (Primrose Bio recommends the Thermo Scientific NTP Set (100 mM), Part # R1481)

AU Cap Analog (if being used in IVT reaction)
Inorganic pyrophosphatase (Recommended) (Primrose Bio recommends Thermo Scientific, Part # EF0221)

RNase inhibitor (Optional) (Primrose Bio recommends Thermo Scientific, Part # EO0382

RNase-free techniques

Ensure all reagents used are 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 nuclease-free reaction vessel.

Recommended IVT Reaction Instructions

1. Thaw 5x IVT buffer, NTPs, linear DNA template and cap analog at room temperature. Mix and pulse-spin in microfuge to collect liquid to the bottom of tubes. Keep at room temperature while in use.

2. Place the ExTend Cap AU RNA polymerase, RNase inhibitor (optional), and inorganic pyrophosphatase (recommended) on ice. Before use, mix and pulse-spin in microfuge to collect liquid to the bottom of tubes.

3. Assemble the IVT reaction 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, vortex the solution briefly. Add the ExTend Cap AU RNA polymerase as the final component to the IVT reaction mixture. Seal the IVT reaction mixture and incubate the IVT reaction at 37°C for 2 hours.

Table 1. Suggested IVT Reaction Conditions for ExTend Cap AU

REAGENT	AMOUNT	FINAL CONCENTRATION IN IVT REACTION
Nuclease-free water	add water up to a final IVT volume of 20 µL	--
5x IVT Reaction Buffer	4 µL	1x
ATP (100 mM)	1.8 µL	9 mM
GTP (100 mM)	1.8 µL	9 mM
UTP (100 mM)	1.8 µL	9 mM
CTP (100 mM)	1.8 µL	9 mM
Cap analog (100 mM)	0.8 µL	4 mM
RNase inhibitor (40 unit/µl)[1]	0.5 µL	1 U/µL
Inorganic Pyrophosphatase (0.1 unit/µl)[2]	0.4 µL	0.002 U/µL
Template DNA[3]	0.9 µL (225 ng)	4 nM
	Vortex briefly	
ExTend Cap AU RNA polymerase	2 µL	400 Units
Total reaction volume	**20 µL**	--

[1] Addition of RNase inhibitor to the reaction is optional but recommended.

[2] Addition of inorganic pyrophosphatase to the reaction is highly recommended.

[3] Volume to be added to the IVT is based upon the 9.5 kb Linearized DNA template (Part # PBST-0.025) provided at 250 ng/µL. This value may change dependent upon the concentration of the DNA template used.