Prima RNApols™ ExTend GMP-Grade[1]

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Use & Handling
Store at -20°C.

Available Products	Catalog Number	Concentration
Prima RNApols™ ExTend RNA Polymerase - 100 µL	PBR3-0.1	200 U/µL
Prima RNApols™ ExTend RNA Polymerase – 10 mL	PBR3-10	200 U/µL

Description
The Prima RNApols™ ExTend polymerase (Prima ExTend) is designed to synthesize long (≥ 5 kb) mRNA products with high integrity and low dsRNA from *in-vitro* transcription (IVT) reactions.

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

Incorporation of Prima ExTend promoter sequence:
The Prima ExTend 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 Prima ExTend 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.

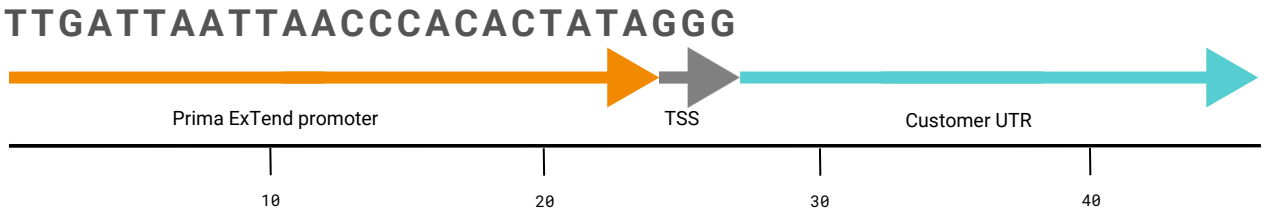

Figure 1: Recommended primer design to incorporate Prima ExTend Promoter sequence

[1] GMP-grade Prima RNApols are manufactured, tested, and released under ISO 13485:2016 certified quality systems, available for clinical and commercial mRNA manufacturing.

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Prima ExTend GMP-Grade IVT Reaction Instructions for Use

Reagent preparation and protocol

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.

Representative IVT Reaction Buffer

A representative 5x IVT reaction buffer is shown in Table 1 for the Prima ExTend RNA polymerase to maximize yield, integrity, and lower dsRNA impurities in the mRNA produced. The buffer can be scaled dependent upon the size of the IVT reaction. Store 5X IVT Reaction Buffer at -20C°.

Table 1: Representative IVT Reaction Buffer Recipe

REAGENT	VOLUME IN 5X REACTION BUFFER	FINAL CONCENTRATION IN 1X *IVT* REACTION BUFFER
Nuclease-free water	4.9 mL	--
1M Tris-HCl, pH 7.5	2.0 mL	40 mM
1M DTT	0.5 mL	10 mM
160 mM Spermidine	0.7 mL	2 mM
5 M NaCl	0.5 mL	50 mM
1M Magnesium Acetate	1.4 mL	28 mM
Total volume of 5x IVT Reaction Buffer	**10 mL**	--

Recommended IVT Reaction Instructions

1. Thaw 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. Prepare IVT reaction buffer and keep at room temperature while in use.

3. Place the Prima ExTend RNA polymerase, RNase inhibitor (optional), and inorganic pyrophosphatase (recommended) on ice.

4. Assemble the IVT reaction at room temperature in the order shown in Table 2. The mixture can be scaled depending upon the number of IVT reactions and the volume of the reaction required.

5. Upon addition of the DNA template to the IVT reaction, vortex the solution briefly. Add the Prima ExTend 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 2: Suggested IVT Reaction Conditions for Prima ExTend

REAGENT	AMOUNT	FINAL CONCENTRATION IN IVT REACTION
Nuclease-free water	add water up to a final IVT volume of 20 µL	--
5x Prima ExTend polymerase *IVT* Reaction Buffer	4 µL	1x
ATP (100 mM)	1 µL	5 mM
GTP (100 mM)	1 µL	5 mM
UTP (100 mM)[1]	1 µL	5 mM
CTP (100 mM)[2]	1 µL	5 mM
Cap analog (100 mM)[3]	0.8 µL	4 mM
RNase inhibitor (40 unit/µl)[4]	0.5 µL	1 U/µL
Inorganic Pyrophosphatase (0.1 unit/µl)[5]	0.4 µL	0.002 U/µL
Template DNA[6]	1.7 µL (450 ng)	8 nM
	Vortex briefly	
Prima RNApol ExTend RNA polymerase	2 µL	400 Units
Total reaction volume	**20 µL**	--

[1] Pseudo-UTP or N1-Methylpseudouridine-5'-Triphosphate can be substituted for UTP as desired

[2] '5-Methylcytidine-5'-triphosphate (5mCTP) can be substituted for CTP as desired'

[3]Addition of cap analog (not provided in the kit) to the reaction is optional.

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

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

[6] This value may change dependent upon the concentration of the DNA template used.

Post-transcriptional options

5' Post-transcriptional capping

The mRNA generated from the Prima RNApols™ ExTend is compatible with the use of post-transcriptional capping systems.