

Prima RNAPols® 7 Pro



DESCRIPTION

Prima RNAPols® 7 Pro is designed to improve yield, reduce dsRNA (>100x), support AG and AU capping strategies, and strong translatability. Prima 7 Pro is compatible with T7 promoters.

USE & HANDLING Store at -20 °C.

THE KIT CONTAINS

COMPONENT	VOLUME	PART NUMBER	CONCENTRATION
Prima 7 Pro	100 µL	PBREC2-0.1	200 U/µL
2 kb Linearized Luc DNA Template	25 µL	PBT1-0.025	250 ng/µL
5x IVT Reaction Buffer	400 µL	PBB3-0.1	5x

DNA TEMPLATE COMPATIBILITY

Prima 7 Pro is compatible with the T7 promoter. The T7 promoter sequence must be present in the DNA template directly upstream of the transcript to be generated to successfully generate mRNA during in vitro transcription reactions. Existing T7-promoter DNA templates can be used without promoter redesign.



Figure 1. Required primer design to utilize Prima 7 Pro. The TSS (AG or AU) follows the promoter and is not part of the promoter sequence.

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.

REAGENTS NOT INCLUDED IN THE KIT

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

PRIMA 7 PRO 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. Store at room temperature while in use.
2. Place Prima 7 Pro, 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 microcentrifuge 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 7 Pro as the final component to the IVT reaction mixture. For optimal results, incubate at 37 °C for 3 hours with 350 rpm agitation.

TABLE 1. SUGGESTED IVT REACTION CONDITIONS FOR PRIMA 7 PRO

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)	8 µL	4 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 7 Pro	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.