



VeraSeq 2.0 High-Fidelity DNA Polymerase

Kit Contents

Part Number	P7511L	P7511S
Concentration	2,000 U/mL	2,000 U/mL
Unit Size	500 U	100 U
5X VeraSeq buffer II	6 X 1.5 mL	1 X 1.5 mL
5X VeraSeq GC buffer	3 X 1.5 mL	1 X 1.5 mL

Polymerase Properties

Extension Rate: 15 seconds per kilobase at 72°C Proofreading (3'-5' exo): Yes, strong Nick-translation (5'-3' exo): No Fidelity: > 50X higher than *Taq* DNA Polymerase Strand Displacement: No Thermostability: Highly thermostable Able to extend an RNA primer: No Extends from a nick: No Generate blunt end products: Yes Uracil read through: No

Common Applications

Ideal choice for applications requiring high fidelity DNA amplication, such as cloning, Next Generation sequencing, synthetic biology.

Protocol

General precautions should be taken when setting up a PCR, including setting up the reaction on ice, adding polymerase last, gentle pipetting, thorough mixing and a quick centrifugation. The following procedure can be used as a guideline. Reactions may need to be optimized individually.

Reaction setup (for 50 μL)*				
Component	Volume (µL)	Final Concentration		
Sterile H ₂ O	х			
5X VeraSeq buffer II or 5X VeraSeq GC buffer ¹	10	1X		
10 mM dNTP mix ²	1	200 μM each		
Primer 1 ³	х	0.2 μΜ		
Primer 2 ³	х	0.2 μΜ		
DNA template ⁴	х	See usage note #4		
VeraSeq 2.0 DNA Polymerase⁵	0.5	1 U		

* Total reaction volume can be adjusted as needed.

Usage Notes:

1. 5X VeraSeq buffer II should be used as the default buffer for high-fidelity amplification. For GC-rich and difficult templates, use 5X VeraSeq GC buffer. 2. VeraSeq 2.0 High-Fidelity DNA Polymerase stalls on uracil residues in the template strand and prevents further extension. Therefore, dUTP should not be used in the reaction. If DNA templates contains uracil or dUTP needs to be incorporated, use VeraSeq ULtra (P7520).

3. A final concentration of 0.2 μ M is recommended for each primer, but it can be varied in the range of 0.2 – 1 μ M.

4. Recommended template quantities:

Complexity	Source Example	Guideline	
Low	Plasmid, Virus, BAC	1 pg – 10 ng	
High	Genomic DNA	50 – 250 ng	

5. One unit is usually sufficient for amplifying most targets. For long targets (>1 kb), difficult templates or to increase yield, it may be necessary to add up to 2 units of enzyme.

6. Both 5X VeraSeq buffer II and GC buffer are formulated to provide a final 1X concentration of MgCl₂ of 1.5 mM. In cases where additional Mg^{2+} is required, adjust the final Mg^{2+} concentration in 0.2 mM steps.

7. For GC rich templates, DMSO may be used to reduce the secondary structure of complex templates. DMSO is generally used at a 3 % final concentration (v/v). If additional optimization is required, adjust the concentration in 1-2% increments (2–9% in final reaction). The primer annealing temperature should be lowered to account for the presence of the solvent.

8. VeraSeq 2.0 High-Fidelity DNA Polymerase is also compatible with other PCR-enhancing additives, such as BSA and betaine.

Typical cycling conditions**						
Step	Temperature	Time	Cycles			
Initial Denaturation	98°C	30 s	1			
Denaturation	98°C	5-10 s				
Annealing	Varies	10-30 s	15-35			
Extension	72°C	15-30 s/kb				
Final Extension	72°C	5-10 min	1			
	4°C	hold				

** Cycling conditions may need to be optimized, depending on the amplicon of interest.

Frequently Asked Questions and Troubleshooting

For Frequently Asked Questions (FAQ) and troubleshooting please visit <u>www.enzymatics.com</u>