

Extender™ DNA Polymerase Blend, 500U

<u>Code</u>	<u>Description</u>	<u>Size</u>
N638-Kit	Extender™ DNA Polymerase Blend, 500U <i>Recommended for amplification of DNA fragments up to 20 kb</i>	1 Kit
	<i>Includes:</i>	
	<i>1 tube of Extender™ Taq DNA Polymerase 500U</i>	
	<i>1 tube of Extender™ Polymerase Buffer A, 10X (without MgCl₂)</i>	
	<i>1 tube of Extender™ Polymerase Buffer S, 10X (with 17.5mM MgCl₂)</i>	
	<i>1 tube of Extender™ Polymerase MgCl₂, 50mM</i>	

General Information:

AMRESCO's Extender™ DNA Polymerase Blend contains a blend of ultra-pure recombinant thermostable DNA polymerases and enhancing factors that are optimized for use in amplification of DNA fragments up to 20kb. The polymerase blend exhibits 3' to 5' proofreading activity, resulting in high fidelity amplifications with error rates two to three times lower than Taq polymerase alone. The polymerase blend has an improved ½ life for enzymatic activity and an increased tolerance for MgCl₂ salt concentrations, pH and template contaminations. Extender™ Taq DNA Polymerase Blend improves amplification results with hard to copy templates such as those containing GC-rich regions, palindromes or multiple repeats. The reactions generate a mixture of blunt end and 3' dA overhangs with a majority of products as blunt ends.

All kit components are tested to be free of endonuclease, exonuclease and non-specific DNase activities.

The kit format provides a choice of buffers for your amplification optimizations. Each kit includes:

- 1 vial of Extender™ Taq DNA Polymerase, 500U
- 1 vial of Extender™ Polymerase Buffer A, 10X (without MgCl₂, and optimized for use with 0.1 – 0.2mM of each dNTP)
- 1 vial of Extender™ Polymerase Buffer S, 10X (with 17.5 mM MgCl₂ and optimized for use with 0.35mM of each dNTP)

Unit Definition:

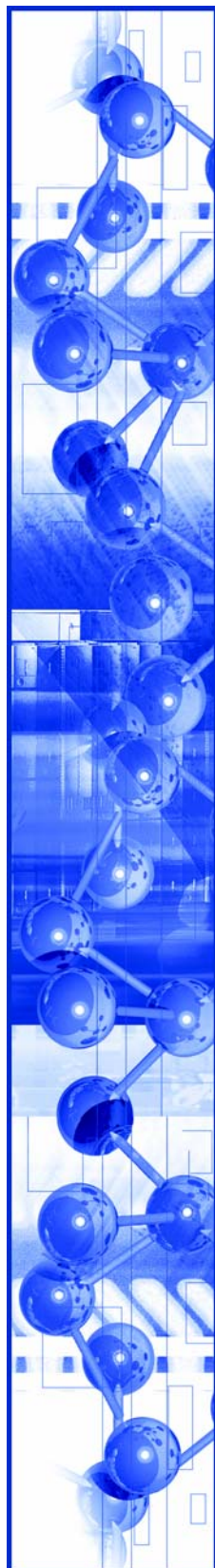
1U is defined as amount of enzyme required to catalyze the incorporation of 10 nmol of dNTP into acid-insoluble material in 30 minutes at 74°C.

Storage/Stability:

Store product at -20°C.

Application Disclaimer

For Research Use Only. Not for Therapeutic or Diagnostic Use.



Reaction Buffers:

Extender™ Polymerase Buffer A, 10X (without MgCl₂): This buffer is optimized for use with 0.1 – 0.2mM of each dNTP. Buffer A contains: 500mM KCl, 100mM Tris-HCl (pH 9.1 at 20°C), 0.1% Triton™ X-100.

Extender™ Polymerase Buffer S, 10X (with 17.5mM MgCl₂): This buffer is optimized for use with 0.35mM of each dNTP. Buffer S contains: 160mM (NH₄)₂SO₄, 500mM Tris-HCL (pH 9.2 at 22°C), 17.5mM MgCl₂ and 0.1% Triton™ X-100.

Storage Buffer:

20mM Tris-HCl (pH 8.0 at 22°C), 100mM KCl, 0.5% Tween™ 20, 0.5% Nonidet-P40, 0.1mM EDTA and 50% glycerol. Store at -20°C.

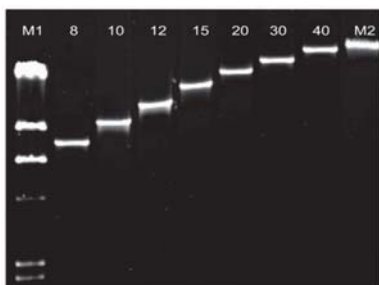
Procedure:
SUGGESTED INITIAL CONDITIONS FOR AMPLIFICATION OF VARIOUS PRODUCT SIZES
Table 1: Reaction Mix Final Concentration

Extender™ Polymerase – 2 U per 50 µl Reaction Mix Primers: 0.2 – 1 uM Template: Plasmid – 0.02 – 2 ng Lambda – 0.1 – 150ng Genomic – 0.05 – 5 ug	Product Size	100bp – 5kb	5kb – 8kb	8ib-20kb
	dNTP Mix	100 µM	200 µM	360 µM
	Extender™ Buffer (1X)	A	A	S
	Ultra Pure DMSO or formamide	-	3%	3%

Table 2: Suggested Initial Amplification Protocol

Product Size	100bp – 5kb	5kb – 8kb	8kb – 20kb
Denaturation	94°C, 2 min	94°C, 2 min	94°C, 2 min
Denaturation	94°C, 30 s	94°C, 12 s	94°C, 12 s
Annealing (primer dependent)	50-68°C, 30 s	50-68°C, 30 s	50-68°C, 30 s
Extension per 1kb	72°C, 30 s	72°C, 45 s	68°C, 1 min
Cycles	25 – 35	25 – 35	25 - 35
Final Extension	72°C, 7 min	72°C, 7 min	68°C, 7 min

0.7% TAE Agarose Gel Showing Amplification With Extender™ DNA Polymerase Blend. Lane M1: Lambda/HindIII Marker; Lane 8: 8kb amplification product from 0.25mM dNTPs, 0.85 U Extender™ Polymerase and 3% formamide; Lanes 10-20: 10kb, 12kb, 15kb and 20kb amplification products using 0.36mM dNTPs and 0.85U Extender™ Polymerase and 3% formamide; Lane 30 and Lane 40: 30kb and 40kb amplification products using 0.36mM dNTPs and 0.85U Extender™ Polymerase and 3% formamide; Lane M2: Lambda DNA at 48kb





Related Products

<u>Code</u>	<u>Product</u>
E0636-40UMoles	dNTP Mixture, 25mM (100mM mixture of 25mM each dNTP)
0658-4L	TBE Buffer, 10X liquid concentrate
0658-500ml	
0796-1.6L,	TAE Buffer, 25X liquid concentrate
0796-4L	
0710-25g,	Agarose I
0710-100g	
0710-500g	
E112-100ml, E112-500ml	TE Buffer, 1X Sterile Solution (pH 8.0)
N313-Kit (5x1ml)	EZ-Vision III, DNA Dye as Loading Buffer
N472-Kit (5x1ml)	EZ-Vision I, DNA Dye as Loading Buffer
E406-5ml	Ethidium Bromide, 0.625 mg/ml Dropper Bottle



www.interchim.com



211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com

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