

Thermo Scientific Extensor Long Range Enzyme Blend

Description:

The Extensor Long Range Enzyme Blend is a blend of ThermoPrime Taq DNA Polymerase and a proprietary proofreading enzyme. The two enzymes act synergistically, forming a mix that can amplify DNA fragments with double the yields of *Pfu* and at least six times higher fidelity than standard *Taq* DNA polymerase. The system can also generate larger products than either enzyme individually (> 20kb). This makes the enzyme mix ideal for the full-length amplification of sequences for cloning.

Kit Contents:

Vial	Pack Size (cap color)			
v iai	А	В		
Extensor Long Range PCR Enzyme	20 µl (clear)	100 µl (clear)		
Extensor Long Range PCR Buffer 1	1.5 ml (red)	1.5 ml (red)		
Extensor Long Range PCR Buffer 2	1.5 ml (green)	1.5 ml (green)		
Extensor Long Range PCR MgCl ₂	1.5 ml (clear)	1.5 ml (clear)		

The Extensor Long Range Enzyme Blend comes supplied with 10X Extensor Buffer 1 (red cap) for PCR < 12kb, 10X Extensor Buffer 2 (green cap) for PCR > 12kb and a vial of 25mM MgCl₂ (clear cap) for any supplementary optimization that may be required. The 10X Buffers both contain 22.5mM MgCl₂.

Storage
Conditions:Store at -20°C in a constant temperature freezer for up to 1 year. Avoid
freeze thawing. Shipped on ice within the UK and on dry ice
internationally and within the US.

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Protocol:

It is recommended to prepare the reaction on ice. Increase enzyme, primer and dNTP concentration for long templates.

Example of reaction mix preparation. Mix together the following:

	Volume reaction	Final Concentration
10X Extensor Buffer 1 or 2 (22.5 mM MgCl ₂) ¹	2.5 µl	1X
Extensor PCR Enzyme Mix $(5U/\mu l)^2$	0.125 µl	0.625 U
dNTP Mix (20 mM) ³	1.25 µl	0.25 mM of each nucleotide
Primer forward (10 µM each)	$0.5 \mu^{14}$	200 nM ⁴
Primer reverse (10 µM each)	0.5 μl ⁴	200 nM ⁴
Water (PCR Grade)	Variable	
DNA Template	0.5-10 µl	1-250 ng
Total Reaction Volume	25 µl	

1 - Use Buffer 1 for amplifications up to 12kb. Use Buffer 2 for amplifications longer than 12kb or problematic amplifications of any length.
2 - For long PCR use 0.25 μl of enzyme in a 25 μl reaction.

For long FCR applications, a final concentration of 350 μM-500 μM of each dNTP is recommended.
 Primers can be used at 0.4 μM for very long extensions.

Templates: For the amplification of large DNA fragments, the quality of the template DNA is very important, as are the denaturation conditions. Keep template DNA denaturation steps as short as possible. Use Extensor Buffer 2 for DNA templates > 12 kb and when difficulties are expected or encountered. 250 ng human genomic DNA is generally sufficient to provide good PCR results. When using simple templates (such as λ DNA), 1-10 ng template DNA should prove sufficient; the number of cycles may be reduced by 5 and Extensor Buffer 1 can be used.

Thermal Cycler Programming:	An example of a long PCR thermal cycling program is given:				
	Initial denaturation	92–94°C ¹	2 min	1 cycle	
	Denaturation Annealing Extension	92–94°C 50–68°C ² 68°C ³	10 sec 30 sec x min ⁴	10 cycles	
	Denaturation Annealing	94°C 50–68°C ²	10 sec 30 sec	15–20 cycles	

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Extension (+10 s/cycle)	68°C3	x min⁴	
Final extension	68°C	7 min	1 cycle

¹ – When amplifying over 15kb, use a denaturation temperature of 92°C. ² – Annealing temperature dependent on primers.

³ – Always use an extension temperature of 68° C, if possible. Often good results are obtained

using a single annealing/extension step at 68°C. ⁴ - Extension times depend on the length of sequence to be amplified (see table below).

Amplicon size (kb)	3	6	10	20	30	40
Extension time (min.)	2	4	8	15	20	30

Ordering	AB-0720/A	Extensor Long Range PCR Enzyme Blend	100 units
Information:	AB-0720/B	Extensor Long Range PCR Enzyme Blend	500 units
	AB-0720/C	Extensor Long Range PCR Enzyme Blend	5,000 units

Troubleshooting

For technical information or troubleshooting contact Thermo Scientific Genomics Tech Support:

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