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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)	
	A	B
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 $MgCl_2$ (clear cap) for any supplementary optimization that may be required. The 10X Buffers both contain 22.5mM $MgCl_2$.

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/µl)[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 µl[4]	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.
3 - For long PCR applications, a final concentration of 350 µM–500 µM of each dNTP is recommended.
4 - 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	92–94°C	10 sec	
Annealing	50–68°C[2]	30 sec	10 cycles
Extension	68°C[3]	x min[4]	
Denaturation	94°C	10 sec	
Annealing	50–68°C[2]	30 sec	15–20 cycles

Extension (+10 s/cycle)	68°C[3]	x min[4]	
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 Information:

AB-0720/A	Extensor Long Range PCR Enzyme Blend	100 units
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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Email	techservice.genomics@thermofisher.com	techservice.emea.genomics@thermofisher.com
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