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Extensor Long Range PCR Kit

Description:

The Extensor Long Range PCR Enzyme Mix 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 approximately 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 (cap color)	Pack Size	
	A	B
Extensor Long Range PCR Enzyme (clear)	20µl	100µl
Extensor Long Range PCR Buffer 1 (red)	1.5ml	1.5ml
Extensor Long Range PCR Buffer 2 (green)	1.5ml	1.5ml
Extensor Long Range PCR $MgCl_2$ (clear)	1.5ml	1.5ml
dNTP Mix (brown)*	0.5ml	1.6ml

*contains 5mM of each dNTP (dATP, dGTP, dCTP and dTTP)

The Extensor Long Range PCR Enzyme Mix comes supplied with 10X Extensor Buffer 1 (red cap) for PCR < 12kb, 10X Extensor Buffer 2 (green cap) for PCR > 12kb, a vial of dNTP Mix (brown cap) 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 for international and within the US.

For Research Purposes Only

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Protocol:

It is recommended that two master mixes are prepared (on ice). Mix dNTPs, primers and template in one master mix; buffer, enzyme and $MgCl_2$ in the other. This eliminates the need for a hot start, as well as avoiding any degradation of primers and template through the proofreading activity present in the Extensor Long Range PCR Enzyme Mix. The use of wax is not recommended, as it prevents adequate mixing of reaction components, leading to low yields. All reaction tubes should be free of DNase and DNA contamination. Increase enzyme, primer and dNTP concentration for long templates.

Mix together the following:

Master Mix 1	**Volume per 25µl reaction**
10X Extensor Buffer 1 or 2 (22.5mM $MgCl_2$)[1]	2.5µl
Extensor Long Range PCR Enzyme Mix (5U/µl)[2]	0.125–0.25µl
$MgCl_2$ (if required for optimization)	x
Distilled water	to 25µl

Master Mix 2	**Volume per 25µl reaction**
20mM dNTPs (5mM of each dNTP)[3]	1–2.5µl
Template	x
Upstream primer (to final conc. of 0.2µM)[4]	x
Downstream primer (to final conc. of 0.2µM)[4]	x
Distilled water	to 25µl

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 0.5µl of enzyme in a 50µl reaction.
3 - For long PCR applications, a final concentration of 350µM–500µM of each dNTP is recommended
4 - Primers can be used at 0.4µM for very long extensions.

Mix both master mixes just prior to reaction cycling.

The following points should also be noted:

- Ensure proper mixing of components and always use thin-walled PCR tubes.
- Use a mineral oil overlay unless a heated lid thermocycler is used.
- Touchdown PCR may increase PCR product specificity.
- For best results, use primers of lengths 22–34 nucleotides with annealing temperatures over 60°C.

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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 > 12kb and when difficulties are expected or encountered. 250ng human genomic DNA is generally sufficient to provide good PCR results. When using simple templates (such as λ DNA), 1–10ng template DNA should prove sufficient; the number of cycles may be reduced by 5 and Extensor Buffer 1 can be used.

Thermal Cycler Programming: For high fidelity PCR, a standard protocol should be used. For long PCR, modifications have to be made. 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

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Ordering Information:

AB-0720/A/N	Extensor Long Range PCR Kit	100 units
AB-0720/B/N	Extensor Long Range PCR Kit	500 units

All sizes are supplied with 10X Reaction Buffer, 20mM dNTP Mix and 25mM $MgCl_2$.

Troubleshooting:

1. *No product detected*
   Try reducing the annealing temperature, increasing the concentration or quality of template, concentration of $MgCl_2$, the number of cycles or improving the purity of primers used.

2. *Spurious bands appearing on electrophoresis gel*
   When non-specific products are amplified, try increasing the annealing temperature (up to a maximum of 68°C) or reducing primer concentration, template concentration or cycle number.

For further troubleshooting, see www.abgene.com/troubleshoot.asp or contact Thermo Fisher Scientific (ABgene) TechSupport at abgene.techsupport@thermofisher.com

UK TechSupport, call +44 (0) 1372 840 410

For all other regions, please contact your local Thermo Fisher Scientific (ABgene) office / distributor.

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