

Extensor Hi-Fidelity PCR Master Mix

Description: The Extensor Hi-Fidelity PCR Master Mix for long and accurate PCR is a ready-to-use enzyme mix, which reduces the risk of contamination and pipetting errors. The enzyme mix can amplify DNA fragments with double the yields of *Pfu* and at least four times higher fidelity than standard *Taq* DNA polymerase. The Extensor PCR Enzyme Mix, dNTPs, Extensor Reaction Buffer (1 or 2) and MgCl₂ are all present in the mix. Furthermore, each mix is available in a ReddyMix™ format, for direct loading onto electrophoresis gels.

Kit Contents:

Cat. No.	Vial	Pack Size	
		A	B
AB-0792	Extensor PCR Master Mix 1	1ml	5 x 1ml
AB-0793	Extensor PCR Master Mix 2	1ml	5 x 1ml
AB-0794	ReddyMix Extensor PCR Master Mix 1	1ml	5 x 1ml
AB-0795	ReddyMix Extensor PCR Master Mix 2	1ml	5 x 1ml

Each vial contains 1.0ml of a 2X working concentration of Extensor Hi-Fidelity PCR Master Mix, which is sufficient for 80 x 25µl reactions. The mix, with the addition of the template and primers in a final reaction volume of 25µl, contains the following:

Cat. No.	Buffer*	dNTPs (µM)	MgCl ₂ (mM)	Total DNA Polymerase	ReddyMix™
AB-0792	1	350 each	2.25	1.25U	No
AB-0793	2	500 each	2.25	1.25U	No
AB-0794	1	350 each	2.25	1.25U	Yes
AB-0795	2	500 each	2.25	1.25U	Yes

* Buffer 1 is used for amplifications up to 12kb.

* Buffer 2 is used for amplifications longer than 12kb or problematic amplifications of any length.

Storage Conditions:

Store at -20°C in a constant temperature freezer for up to 1 year. The vial can be stored at 4°C for up to 1 month. Avoid freeze thawing. Shipped on ice within the UK and on dry ice for international and within the US.

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Protocol: For a 25µl reaction, take 12.5µl of Extensor Master Mix and add template, primers and water in a 12.5µl volume (scale up or down accordingly if required). Generally, 100–250ng template DNA, and 200nM (final concentration) of each primer is added. It is recommended that the Extensor Master Mix and added components are kept on ice. This removes the need for a hot start, as well as avoiding any degradation of primers and template through the 3' to 5' proofreading activity present in the Extensor Master 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 sterile and certified DNase/RNase free. The following points should also be noted:

- The Extensor Hi-Fidelity PCR Master Mix offers very robust amplification up to 15kb of human genomic DNA. Between 15kb and 20kb, more optimization may be required.
- Ensure proper mixing of reaction 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.
- Primers can be used at 400nM 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 ≥ 12kb and when difficulties are expected or encountered. 125ng 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.

Tip: The gel precipitant in ReddyMix™ Master Mix causes a slight increase in the thermal mass of the reaction mix. In a small number of cases this may necessitate some minor re-optimization of the thermal cycler program. If this is the case we suggest decreasing the temperature of the annealing step by 1–2°C.

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	68°C ³	x min ⁴	(+10s/cycle)
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

Troubleshooting:

1. *No product detected*
Try reducing the annealing temperature, increasing the concentration or quality of template, concentration of MgCl₂, 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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