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Thermo Scientific 2X Extensor Long Range PCR Master Mix

Description: The Extensor Long Range 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 six times higher fidelity than standard *Taq* DNA polymerase. The Extensor PCR Enzyme Blend, dNTPs, Extensor Reaction Buffer (1 or 2) and $MgCl_2$ 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 w/ Buffer 1	1 ml	5 x 1 ml
AB-0793	Extensor PCR Master Mix w/ Buffer 2	1 ml	5 x 1 ml
AB-0794	ReddyMix Extensor PCR Master Mix w/ Buffer 1	1 ml	5 x 1 ml
AB-0795	ReddyMix Extensor PCR Master Mix w/ Buffer 2	1 ml	5 x 1 ml

Each vial contains 1.0ml of a 2X working concentration of Extensor Long Range 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.

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INFORMATION

Storage Conditions

Store at -20°C until ready for use. Extensor Long Range PCR Master Mix is stable for a minimum of 12 months. Avoid repeated freeze thawing. Shipped on ice within the UK and on dry ice internationally and within the US.

DNA Template

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.

Tips before use

- It is recommended that the Extensor Master Mix and added components are kept on ice.
- The Extensor Long Range PCR Master Mix offers very robust amplification up to 15kb of human genomic DNA. Between 15kb and 20kb, more optimization may be required.
- Touchdown PCR may increase PCR product specificity.
- Primers can be used at 400nM for very long extensions.

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PROTOCOL

Example of reaction mix preparation.
The volume of each component is for a **25 µl final reaction.**

Reaction Mix: Mix gently and spin down the master mix prior to use.

	Volume	Final Conc. 1X
Extensor PCR Master Mix	12.5 µl	1X
Primer forward (10µM each)	0.5 µl*	200nM *
Primer reverse (10µM each)	0.5µl*	200nM *
Water (PCR Grade)	Variable	
DNA Template	0.5 – 10 µl	100 – 250 ng
Total volume	25 µl	

*Scale up or down the volume and concentration as appropriate

Example of a **long PCR thermal cycling program**:

	Temp.	Time	Number of cycles
Initial denaturation	92–94°C[1]	2 min	1 cycle
Denaturation	92–94°C	10 sec	10 cycles
Annealing	50–68°C[2]	30 sec	
Extension	68°C[3]	x min[4]	
Denaturation	94°C	10 sec	15–20 cycles (+10s/cycle)
Annealing	50–68°C[2]	30 sec	
Extension	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

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.

Troubleshooting

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

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