

2x Pre-Aliquoted Extensor Hi-Fidelity PCR Master Mix, 25µl Reaction

Description: Extensor Hi-Fidelity PCR Master Mix is a pre-aliquoted ready-to-use enzyme mix for long and accurate PCR without the need to mix individual components, reducing the risk of contamination and pipetting errors. The Extensor PCR Enzyme Mix, dNTPs, Extensor Reaction Buffer and MgCl₂ are all present in the mix.

Ordering Information: Select the buffer type from Table 1 and consumable type and size from Table 2.

| Cat. No. | Buffer Type |
|-----------|---|
| EX1-xxx-6 | Buffer 1 - For templates <12kb |
| EX2-xxx-6 | Buffer 2 - For templates >12kb or problematic amplifications of any length. |

Note: xxx denotes plastic consumable type

| Cat. No. | Description | Quantity |
|-------------|--|--------------------------------------|
| EX?-266-6/a | 0.2ml Thermo-Strips & Domed Caps | 25µl PCR x 12 strips of 8 tubes |
| EX?-266-6/b | 0.2ml Thermo-Strips & Domed Caps | 25µl PCR x 10 x 12 strips of 8 tubes |
| EX?-337-6/a | Individual 0.2ml Domed Cap Thermo-Tubes | 25µl x 96 tubes |
| EX?-337-6/b | Individual 0.2ml Domed Cap Thermo-Tubes | 25µl x 10 x 96 tubes |
| EX?-350-6/a | Individual 0.5ml Flat Cap Thermo-Tubes | 25µl x 96 tubes |
| EX?-350-6/b | Individual 0.5ml Flat Cap Thermo-Tubes | 25µl x 10 x 96 tubes |
| EX?-384-6 | Thermo-Fast [®] 384 Plates | 25µl x 10 plates of 384 wells |
| EX?-489-6/a | Individual 0.5ml Domed Cap Thermo-Tubes | 25µl x 96 tubes |
| EX?-489-6/b | Individual 0.5ml Domed Cap Thermo-Tubes | 25µl x 10 x 96 tubes |
| EX?-600-6 | 0.2ml Thermo-Fast [®] 96 Plates | 25µl x 10 plates of 96 wells |
| EX?-620-6/a | Individual 0.2ml Flat Cap Thermo-Tubes | 25µl x 96 tubes |
| EX?-620-6/b | Individual 0.2ml Flat Cap Thermo-Tubes | 25µl x 10 x 96 tubes |
| EX?-700-6 | 0.2ml Low Profile Thermo-Fast [®] 96 Plates | 25µl x 10 plates of 96 wells |
| EX?-772-6/a | 0.2ml Low Profile Thermo-Strips & Domed Caps | 25µl PCR x 12 strips of 8 tubes |
| EX?-772-6/b | 0.2ml Low Profile Thermo-Strips & Domed Caps | 25µl PCR x 10 x 12 strips of 8 tubes |
| EX?-773-6/a | 0.2ml Low Profile Thermo-Strips & Flat Caps | 25µl PCR x 12 strips of 8 tubes |
| EX?-773-6/b | 0.2ml Low Profile Thermo-Strips & Flat Caps | 25µl PCR x 10 x 12 strips of 8 tubes |
| EX?-800-6 | 0.2ml Skirted Thermo-Fast [®] 96 Plates | 25µl x 10 plates of 96 wells |
| EX?-848-6/a | 0.2ml Low Profile Thermo-Strips & Domed Caps | 25µl PCR x 8 strips of 12 tubes |
| EX?-848-6/b | 0.2ml Low Profile Thermo-Strips & Domed Caps | 25µl PCR x 10 x 8 strips of 12 tubes |
| EX?-849-6/a | 0.2ml Low Profile Thermo-Strips & Flat Caps | 25µl PCR x 8 strips of 12 tubes |
| EX?-849-6/b | 0.2ml Low Profile Thermo-Strips & Flat Caps | 25µl PCR x 10 x 8 strips of 12 tubes |

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| Cat. No. | Description | Quantity |
|-----------------|---|--------------------------------------|
| EX?-900-6 | 0.2ml Semi Skirted Thermo-Fast [®] 96 Plates | 25µl x 10 plates of 96 wells |
| EX?-937-6 | Thermo-Fast [®] 384 Mark II Plates | 25µl x 10 plates of 384 wells |
| EX?-990-6 | 0.2ml Ultra Rigid Semi Skirted Thermo-Fast [®] 96 Plates | 25µl x 10 plates of 96 wells |
| EX?-1000-6 | 0.2ml Ultra Rigid Skirted Thermo-Fast [®] 96 Plates | 25µl x 10 plates of 96 wells |
| EX?-1100-6 | 0.2ml Thermo-Fast [®] 96 PCR Detection Plate | 25µl x 10 plates of 96 wells |
| EX?-1111-6 | Thermo-Fast [®] 384 Diamond PCR Plate | 25µl x 10 plates of 384 wells |
| EX?-1113-6/a | 0.2ml Thermo-Strips with Domed Caps | 25µl PCR x 8 strips of 12 tubes |
| EX?-1113-6/b | 0.2ml Thermo-Strips with Domed Caps | 25µl PCR x 10 x 8 strips of 12 tubes |
| EX?-1114-6/a | 0.2ml Thermo-Strips with Flat Caps | 25µl PCR x 8 strips of 12 tubes |
| EX?-1114-6/b | 0.2ml Thermo-Strips with Flat Caps | 25µl PCR x 10 x 8 strips of 12 tubes |

Note: Replace ? with buffer type

Example: **EX1-337-6/a**
2x Extensor Hi-Fidelity PCR Master Mix with Buffer 1, (Reaction Volume 25µl) Pre-aliquoted into 96 x 0.2ml Domed Cap Thermo-Tubes

Kit Components: Each well contains 12.5µl of a 2x working concentration PCR Master Mix. The addition of the template and primers (in a volume of 12.5µl) results in a final reaction volume of 25µl, containing:

Buffer 1

1x Extensor Buffer 1
 1.25 units DNA Polymerase
 2.25mM MgCl₂
 350µM each of dATP, dCTP, dGTP and dTTP

Buffer 2

1x Extensor Buffer 2
 1.25 units DNA Polymerase
 2.25mM MgCl₂
 500µM each of dATP, dCTP, dGTP and dTTP

Thermo-Fast[®] 96 plates are provided capped with Domed Cap Strips. An extra set of caps for application after the addition of template and primers is included with both plates and Thermo-Strips. Thermo-Fast[®] 384 plates are supplied heat sealed with Easy Peel (or Diamond Foil Seal for Diamond plates).

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Overview of 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. The enzyme mix has a fidelity that is at least four times higher than standard *Taq* DNA polymerase. 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. Above 15kb, more optimisation 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 (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.

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Thermal Cycler Programming:

For high fidelity PCR, a standard protocol should be used. For long PCR, modifications may need to be made. An example of a long PCR thermal cycling programme is given:

| | | | |
|------------------------|----------------------|---------------------|--------------|
| Initial denaturation | 92-94°C ¹ | 2 min | 1 cycle |
| Denaturation | 92-94°C | 10 sec | |
| Annealing ² | 50-68°C | 30 sec | 10 cycles |
| Extension | 68°C ³ | x min ⁴ | |
| Denaturation | 94°C | 20 sec | |
| Annealing ² | 50-68°C | 30 sec | 15-20 cycles |
| Extension | 68°C | x min (+20 s/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 |

After PCR, a sample (10–30% of reaction) may be loaded directly on a gel.

Troubleshooting: 1 *No product detected*

Try reducing the annealing temperature, increasing the concentration or quality of template, 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 template concentration or cycle number.

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Storage Conditions: Can be stored at -20°C in a constant temperature freezer for up to 1 year. Avoid freeze thawing. Once opened, the vial can be stored at 4°C for up to 1 month. Shipped on ice.

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