# 2X Hyperfusion plus master mix

## Product description

Our product 2X Hyperfusion plus master mix offers superior performance for major PCR applications with high fidelity, powerful amplification ability and faster extension speed. Hyperfusion plus DNA polymerase is composed of a DNA-binding domain fused to a Pyrococcus-like proofreading polymerase. Its unique structure, a novel Pyrococcus-like enzyme fused with a processivity-enhancing domain, increases fidelity and speed. The high fidelity makes hyperfusion plus DNA polymerase a superior choice for cloning or other subsequent applications. Hyperfusion plus DNA polymerase possesses one of the most accuracy with an error rate that is 50-fold lower compared to Taq DNA Polymerase and 6-fold lower than Pyrococcus Furiosus DNA Polymerase (Pfu).

Hyperfusion plus DNA polymerase possesses 5′→ 3′ polymerase activity and 3′→ 5′ exonuclease activity. It will generate blunt-ended products in the amplification products without an A overhang which appears in the product amplified with Taq polymerase. The polymerase has been capable of amplifying genomic fragment as long as 20.1 kb in our assays. In PCR reaction, elongation rate of hyperfusion plus DNA polymerase is about 15-30 sec/kb depending on the complexity of the amplicon.

2X Hyperfusion plus master mix is a ready-to-use 2×premix solution containing polymerase, dNTPs and an already optimized buffer system.

## Components and storage conditions

| Components | K1119-1 mL | K1119-5x1 mL | K1119-10x1 mL |
|---|---|---|---|
| 2X Hyperfusion plus master mix | 1 mL | 5 mL | 10 mL |
| Store the components at -20°C for 12-24 months. | | | |

## Experimental manipulation

1. **Set up the appropriate reactions on ice.**

   We recommend setting all reaction components on ice. Experimenters should carefully thaw the tube before opening and ensure homogeneity.

   It is intensively recommended that the 2X Hyperfusion plus master mix should be added to the PCR

mixture at last, since the enzyme exhibits 3′→5′ exonuclease activity that can degrade primers in the absence of dNTPs.

Please pay attention to protocols with 2X Hyperfusion plus master mix that may differ from protocols with other standard polymerases due to the nature of hyperfusion plus DNA Polymerase.

| COMPONENT | 20 µl REACTION | 50 µl REACTION | FINAL CONCENTRATION |
|---|---|---|---|
| ddH2O | add to 20 µL | add to 50 µL | |
| 10 µM Forward Primer | 0.8 µL | 2 µL | 0.4 µM |
| 10 µM Reverse Primer | 0.8 µL | 2 µL | 0.4 µM |
| Template | variable | variable | < 250 ng |
| 2X Hyperfusion plus master mix | 10 µL | 25 µL | |

A. The recommended final primer concentration is 0.4 µM, but it can be varied in a range of 0.2 - 1.0 µM, you can adjust the concentration. Oligonucleotide primers are generally recommended as a length between 20 - 40 bp and ideally having a GC content of 40 - 60%.

B. Use of high quality, purified DNA templates could greatly improve the success rate of PCR. For low complexity DNA (e.g., plasmid, viral, λ or BAC DNA), DNA template amount can be 1 pg - 10 ng per 50 µL reaction volume. For high complexity genomic DNA, the amount of DNA template should be 50 - 250 ng per 50 µL reaction volume. If the template DNA is obtained from a cDNA synthesis reaction, the volume of the template should not exceed 10% of the final PCR reaction volume.

2. **Gently mix the reaction and spin down in microcentrifuge.**

Mix and briefly centrifuge the reaction tube, then transfer the reactions to a thermocycler preheated to the denaturation temperature (98°C).

If the thermocycler does not have a heated lid, overlay the sample with mineral oil.

3. **PCR setup parameters**

| | Temperature | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 98°C | 30 sec-1min | 1 |
| Denaturation | 98°C | 10 sec | variable |
| Annealing | 55-58°C | 15-30 sec | |
| Extension | 72°C | 15–30sec per kb | |
| Final extension | 72°C | 2min | 1 |
| Hold | 4°C | +∞ | 1 |

A. For template length ≤10 kb, we recommend 30 sec initial denaturation at 98°C. For template length ≥ 10 kb, we recommend 1 min of initial denaturation at 98°C.

B. The optimal annealing temperature for Hyperfusion plus DNA Polymerase might differ significantly from that of Taq-based polymerases. The Hyperfusion plus DNA Polymerase could stabilize primer-template hybridization. As generally recommended, anneal for 15-30 seconds at 55

- 58°C. If you can't generate an ideal product or yield, use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination.

C. Extension time depends on amplicon length and complexity. For low complexity DNA (e.g. plasmid, viral, λ or BAC DNA) use an extension time of 15 seconds per kb. For high complexity genomic DNA 30 seconds per kb is recommended. For some cDNA templates, the extension time can be increased up to 40 seconds per kb to obtain optimal results. For generally use, 20 second per kb is recommended.

4. **Electrophoresis**

If needed to subject the PCR product to agarose gel electrophoresis, our product SYBR Safe DNA Gel Stain (Cat: A8743) or Hyper Gel Red（Cat: A8746）is available.

## Notes

1. Hyperfusion plus DNA Polymerase annealing temperature is different from many common DNA polymerases (such as Taq DNA polymerase). If you can't generate ideal experimental results under this temperature, you may set gradient annealing temperature to optimize the experimental conditions.
2. Use 15 - 30 sec/kb for extension. Do not exceed 1 min/kb.
3. Hyperfusion plus DNA Polymerase produces blunt end DNA products.
4. Hyperfusion plus DNA Polymerase has a powerful polymerization ability. Experimenters should operate on ice during the whole experiment of PCR, otherwise the enzyme is active at room temperature. It is possible to polymerize primers to form primer dimers under room temperature, which cause the depletion of the primers, thus the PCR efficiency will decrease.
5. The PCR products generated by using Hyperfusion plus DNA Polymerase have blunt ends. If cloning is the next step, then blunt-end cloning is recommended. If T/A-cloning is preferred, then DNA should be purified prior to A-addition (Any remaining hyperfusion plus DNA Polymerase will degrade the A overhangs, creating blunt ends again.). Addition of an untemplated -dA can be done with Taq DNA Polymerase or Klenow exo–. Experimenters could incubate the purified PCR product with 1x Taq buffer, 2.5 mM MgCl2, 0.2 mM dATP and 1 U Taq DNA polymerase in 10 µL reaction mixture up to 30 min at 72 °C.
6. This product is for scientific purposes only.

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