2X Hyperfusion plus master mix (With dye)

Product description

Our product 2X Hyperfusion plus master mix (With dye) provides 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 detection 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 (With dye) is a ready-to-use 2×premix solution containing polymerase, dNTPs, an already optimized buffer system and dye. Our mix is ideal for any detection and the PCR product can be directly electrophoresed after the amplification without the need to add a loading buffer.

If the subsequent experiment is cloning, product 2X Hyperfusion plus master mix (Cat. No K1119) is your better choice which doesn't contain a dye.

Components and storage conditions

Components	K1117-1 mL	K1117-5x1 mL	K1117-10x1 mL
2X hyperfusion plus master mix (With dye)	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 (With dye) 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（With dye）	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-1 min	1
Denaturation	98°C	10 sec	25-35 cycles (variable)
Annealing	55-58°C	15-30 sec	
Extension	72°C	15–30 sec per kb	
Final extension	72°C	2 min	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℃.

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. This product is for scientific purposes only.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com