Hyperfusion plus DNA polymerase

Product description

Our product high-fidelity HyPerFUsion Plus DNA polymerase provides superior performance for major PCR applications with high fidelity, powerful amplification ability and faster amplification 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. 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. Our HyPerFUsion Plus DNA polymerase is capable of amplifying genomic fragment as long as 20.1 kb in our assays.

This product added an enhancer to hyPerFUsion® high fidelity DNA polymerase (K1032) for higher yields and longer amplification products.

Components and storage conditions

Components	Concentration
Hyperfusion plus DNA polymerase	1,000 units/mL
5X HyPerFUsion Plus buffer (Contain Mg^{2+})	5X
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 mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery. HyPerFUsion Plus DNA polymerase should be pipetted carefully and gently because of the storage buffer containing a high concentration of glycerol.

   We strongly recommend the HyPerFUsion Plus DNA polymerase added to the PCR mixture in the last, since the enzyme exhibits 3′→5′ exonuclease activity which can degrade primers in the absence of dNTPs.

Please pay attention to protocols with HyPerFUsion Plus DNA polymerase 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	
5XHyPerFUsion Plus buffer	4 µL	10 µL	1X
2.5 mM dNTPs	1.6 µL	4 µL	200 µM each
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
HyPerFUsion Plus DNA polymerase (1 U /µL)	0.4 µL	1 µL	0.02 U/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 between20–40 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 reactions. 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.

C. The optimal amount of polymerase depends on the amount of template and the length of the PCR product. Usually, 1 U of hyperfusion plus DNA Polymerase per 50 µL reaction volume will lead to good results, but the optimal amount can range from 0.5 to 2 U per 50 µL reaction depending on the amplicon length and complexity. It is not recommended to exceed 2 U/50 µL (0.04 U/µL).

D. High quality dNTPs should be used for optimal amplification with hyperfusion plus DNA Polymerase. The polymerase cannot read dUTP-derivatives or dITP in the template strand so the use of these analogues or primers containing them is not recommended.

E. Mg^{2+} has been added into the buffer, experimenters don't need to add Mg^{2+} repeatedly. If the chelators (e.g., EDTA) are present, it might be necessary to increase the Mg^{2+} concentration in addition.

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

Experimenters should quickly 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℃	30 sec-1 min	1
Denaturation	98℃	10 sec	variable
Annealing	55-58℃	15-30 sec	
Extension	72℃	15–30 sec per kb	
Final extension	72℃	2 min	1
Hold	4℃	+∞	1

A. For template length≤10 kb, we recommend 30 sec initial denaturation at 98°C. For template length ≥ 10kb, 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. Always use the Tm calculator and instructions to determine the Tm values of primers and optimal annealing temperature. The HyPerFUsion Plus DNA polymerase could stabilize primer-template hybridization. As generally recommended, anneal for 15–30 seconds at 55-58°C. If necessary, 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. The annealing rules for hyperfusion plus DNA polymerase are different from many common DNA polymerases (such as Taq DNA polymerases).
2. Use 15–30 s/kb for extension. Do not exceed 1 min/kb.
3. Use hyperfusion plus DNA polymerase at 0.5–2.0 U per 50 µL reaction volume. Do not exceed 2 U/50 µL.
4. Use 200 µM of each dNTP. Do not use dUTP.
5. Hyperfusion plus DNA polymerase produces blunt end DNA products.
6. 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. Experimenters could incubate the purified PCR product with 1x Taq buffer, 2.5 mM $MgCl_2$, 0.2 mM dATP and 1 U Taq DNA polymerase in 10 μL reaction mixture up to 30 min at 72 °C.

7. This product is for scientific purposes only.

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