SuperGold™ 1×sPfu MasterMix

Description

SuperGold™ 1×sPfu MasterMix contains SuperGold™ sPfu DNA Polymerase (sPfu), dNTPs, dyes and buffers required for PCR reactions. It is a ready-to-use PCR amplification reagent. You only need to add primers and DNA template to perform PCR amplification. The total volume of primers and template added can be varied between 1 and 10μl, which provides strong adjustability. The mastermix can save experimental time and avoid contamination caused by multiple additions of PCR reagents. The mastermix already contains dyes, so the sample can be directly loaded for the electrophoresis after the amplification. The amplified product has blunt ends.

SuperGold™ sPfu DNA Polymerase has extremely high fidelity, which is 69 times as high as Taq DNA polymerase. Extremely high fidelity makes it possible to clone ultra-long fragments. And simple templates such as plasmids and lambda DNA can be amplified by SuperGold™ High Fidelity PCR MasterMix with an effective length of 40 kb, genomes can be amplified with an effective length of 20 kb, and difficult fragments such as cDNA can be amplified with an effective length of 10 kb. In addition, the extension rate of SuperGold™ High Fidelity DNA polymerase can reach as fast as 15 sec/kb, which is four times as high as Taq DNA polymerase.

This product can be applied to perform high-fidelity PCR amplification, colony PCR, blunt-end PCR product amplification, and RT-PCR.

Features

- **High fidelity:** ~ 69 times as high as Taq DNA polymerase
- **High extension rate:** ~ 4 times as high as Taq DNA polymerase
- **Ultra-long fragments:** >40 kb for simple templates (plasmids and lambda DNA), >20 kb for genomes, and >10 kb for cDNA

Protocol

Setting up PCR reaction

Add the components according to the table below, mix thoroughly and then centrifuge for a few seconds:

Composition	Volume for 25μl reaction	Volume for 50μl reaction
Forward Primer (10μM)	0.5 μl	1 μl

Reverse Primer (10μM)	0.5 μl	1 μl
DNA template*	1-4 μl	1-8 μl
SuperGold™ 1×sPfu MasterMix	Make up the total volume to 25μl	Make up the total volume to 50μl

**For simple templates (plasmid, virus, λ and BAC DNA, etc.), add 10-100ng template to 50μl mix; for complex templates, add 100-500ng template to 50μl reaction mix; for cDNA templates, it should not be more than 1/10 of the PCR reaction mix; for RT product templates, only add 2-3μl in the 50μl PCR reaction mix, do not exceed 5μl.*

Thermocycling Conditions for PCR (Recommended)

Step	Temperature	Time	Cycles
Initial Denaturation	98°C	2 min	1
Denaturation*	98°C	5-10 sec	25-40
Annealing**	50-72°C	10 sec	
Extension***	72°C	10-30 sec/kb	
Final Extension	72°C	5 min	1

** The duration of denaturation at 98°C can be set for 5-10 seconds, 5 seconds for simple templates, and 10 seconds for complex templates.*

*** The annealing temperature of the primers is the lower Tm-5 of the two primers, and the annealing time can be set to 10 seconds. When the Tm values of the two primers ⩾70°C, and long primers are used, you can use two-step method for amplification. The annealing temperature and extension temperature in the two-step method are both 72°C.*

**** The extension time depends on the length and complexity of the amplified product. For simple templates such as plasmids and BAC, an extension rate of 15 sec/kb can be used, and for high-complexity genomic DNA, an extension rate of 30 sec/kb can be used. When amplifying products below 1kb, the extension time should not exceed 40 seconds.*

Storage

The minimum shelf life is 1 year at -20°C.