2× S705 HiFi Master Mix

REF: EG24110-S/M/L

Storage Condition

-20℃

Components

Component	EG24110S	EG24110M	EG24110L
2× S705 HiFi Master Mix	1 ml	5×1 ml	20×1 ml
2.5× PCR Enhancer	1 ml	1 ml	5×1 ml

Description

The 2× S705 HiFi Master Mix is a ready-to-use 2× premix that contains S705 High-Fidelity DNA Polymerase, dNTPs, and a optimized reaction buffer for PCR. It only requires the addition of template, primers, and Nuclease-Free Water to perform high-fidelity PCR amplification. S705 High-Fidelity DNA Polymerase is a high-fidelity DNA polymerase obtained through directed evolution.Its fidelity is 70-fold higher than that of Taq polymerase, and its an amplification speed is five times faster than that of Pfu polymerase. The 2× S705 HiFi Master Mix is formulated with unique extension factors and specificity enhancers, which significantly improves the capacity for long fragment amplification, specificity, and product yield. When using simple templates such as λDNA and plasmids, the 2× S705 HiFi Master Mix can easily amplify fragments up to 20 kb in length. When using complex templates like genomic DNA, it can effectively amplify fragments up to 12 kb. Furthermore, the 2× S705 HiFi Master Mix demonstrates good tolerance to PCR inhibitors, enabling excellent amplification even from crude templates.

Application

This product is suitable for PCR amplication using genomic DNA, cDNA, plasmids, and crude extracts as templates.

Protocol

1. Reaction System

Reagent	Amount
2× S705 HiFi Master Mix	25 μl
2.5× PCR Enhancer (optional)	20 μl[a]
Forward primer (10 μM)	1 μl
Reverse primer (10 μM)	1 μl
Template	x μl
ddH_2O	Up to 50 μl

a. When the template with a GC content >60%, it is recommended to add 2.5× PCR Enhancer to optimize the PCR amplification. The recommended program is Touchdown PCR.

2. Recommended template amounts and reaction System

(1) Template amount:

Templates types	Recommended amount
Genomic DNA	10~200 ng
plasmid or viral DNA	10 pg~50 ng
cDNA	1~5 μl (less than 10% of the total reaction volume)
crude template	1~5 μl (less than 10% of the total reaction volume)

(2)Program:

① Three-step:

Step	Temperature	Time
Initial Denaturation[a]	95℃	3~5 min
Denaturation	95℃	10 s
Annealing[b]	55~72℃	15 s
Extension[c]	72℃	30 s/kb
Final Extension	72℃	5 min

30~35 Cycles

a. An initial denaturation of 30~60 seconds is sufficient for most templates; for complex templates, such as GC-rich sequences, it is recommended to extend the initial denaturation time to 3~5 minutes. E. coli colony PCR: It is recommended to adjust the pre-denaturation time to 10 min, enabling efficient amplification without pre-lysis. Yeast colony PCR: No need to adjust the pre-denaturation time, but pre-lysis of yeast is required. Recommended lysis methods: Add NaOH to yeast to a final concentration of 20 mM, then pre-lyse at 95℃ for 10 min; or repeatedly freeze-thaw yeast 2~5 times using liquid nitrogen.

b. Set the annealing temperature according to the Tm value of the primers. If the Tm value of the primers is ≥72℃ , the annealing step can be omitted, allowing the protocol to proceed directly to the extension step (two-step PCR). If necessary, A temperature gradient can also be used to optimize the annealing temperature for each primer pair. Additionally, the annealing temperature has a direct impact on the specificity of amplification. If poor amplification specificity is observed, it may be beneficial to increase the annealing temperature accordingly.

c. For most templates, an extension time of 30 s/kb is adequate for effective amplification; However, for some complex templates, the extension time may be extended to 30~60 s/kb.

② Two-step[d]:

Step	Temperature	Time
Initial Denaturation	95℃	3~5 min
Denaturation	95℃	10 s
Annealing & Extension	65~68℃	30 s/kb
Final Extension	72℃	5 min

30~35 Cycles

d. In general, there is no significant difference between the two-step and three-step programs for PCR amplification. However, for some complex templates (such as long template, uneven Tm distribution, or templates with special structures), it is recommended to the two-step program or Touchdown PCR. Furthermore, when using plasmids as templates for site-directed mutagenesis, the two-step program is also recommended.

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③ **Touchdown PCR[e]:**

Step	Temperature	Time
Initial Denaturation	95℃	3~5 min
Denaturation	95℃	10 s
Annealing	68℃ (-0.2℃ /cycle)	15 s
Extension	72℃	30 s/kb
Final Extension	72℃	5 min

30~40 Cycles

e. This program is for reference only. Touchdown PCR offers various protocols that can be flexibly adjusted according to the experimental requirements.

Notice

1. Please do not use primers and templates containing uracil, or dUTP.

2. S705 High-Fidelity DNA Polymerase has strong proof-reading activity, and its amplification products have blunt ends. If T/A-cloning is the next step, the amplification products should be purified prior to A-addition.

3. To improve the success rate and yield of amplification, please use high-quality templates.

FAQ & Troubleshooting

Problem	Possible Reason	Solution
No amplification products or low yield	Primer	Optimize primer design.
	Annealing temperature	Set temperature gradient and find the optimal annealing temperature.
	Primer concentration	Increase the concentration of primers properly.
	Extend time	increase the extension time to 30~60 sec/kb properly.
	Cycles	Increase the number of cycles to 36~ 40 cycles.
	Template purity	Use templates with high purity.
	Template amount	Adjust the template amount according to the recommended amount and increase it properly.
with impurity bands or diffuse bands	Primer	Optimize primer design.
	Annealing temperature	Try to increase the annealing temperature and set temperature gradient.
	Primer concentration	Decrease the concentration of primers properly.
	Cycles	Decrease the number of cycles to 25~30 cycles.
	Template purity	Use templates with high purity.
	Template amount	Adjust the template amount according to the recommended amount and increase it properly.

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