

Servicebio[®] 2x Fast Pfus PCR Master Mix

Cat. #: G3305

Product Information

Product Name	Cat. No.	Spec.
2 x Fast Pfus PCR Master Mix	G3305-01	1 mL
	G3305-05	5×1 mL

Product Description/Introduction

This Fast Pfus PCR Master Mix is a 2×concentrated solution of optimized Pfu DNA polymerase, dNTPs and all other components required for PCR, expected DNA template and primers. This pre-mixed formulation saves time and reduces contamination due to a reduced number of pipetting steps during PCR set up. The optimized Pfu DNA polymerase ensure efficient PCR reaction, high fidelity, and with quick extension speed of 5-15s/kb. The PCR product are blunt end convenient for downstream experiments and can be direct loading on gel with DNA loading dye.

Storage and Shipping Conditions

Ship with wet ice; Store at 4°C for periods up to 12 months. For longer periods, store at -20°C.

Product Contents

Component	G3305-01	G3305-05
2 x Fast Pfus PCR Master Mix	1 mL	5×1 mL
Manual	One copy	

Assay Protocol / Procedures

1. Gently vortex and briefly centrifuge PCR Master Mix(2×) after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each reaction according to your desired reaction volume.

Component	20 μL rxn	50 μL rxn	Final Concentration
Template ^a	Variable	Variable	as required
Forward Primer (10 μM) ^b	0.8 μL	2 μL	0.4 μM
Reverse Primer (10 μM) ^b	0.8 μL	2 μL	0.4 μM
2×Fast Pfus PCR Master Mix	10 μL	25 μL	1×
(DMSO, optional) ^c	(0.6 μL)	(1.5 μL)	(3%)
Water, nuclease-free	Add to 20 μL	Add to 50 μL	

a: Higher amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification. If the template is plasmid or from bacteriophage, 50ng-5pg added in 50 μL rxn is recommended. If template is genomic DNA, 250ng-50ng added in 50 μL rxn is recommended. If template is cDNA, dilute your original cDNA by 2-100 times, and the volume of diluted cDNA added to reaction is no more than 10% of total reaction volume. If template is culture liquid, the volume added to reaction is no more than 10% of total reaction volume.

b: The appropriate primer concentration is between 0.2-1.0 μM, and 0.4 μM is recommended.

c: For high GC content template, DMSO with no more than 10% total volume can be added to improve PCR efficiency.

3. Gently vortex the samples and spin down.
4. Perform PCR using the recommended thermal cycling conditions below:

Step	Temperature	Time	Number of Cycles
Initial Denaturation ^a	98°C	30 s-120 s	1
Denaturation	98°C	5-10 s	25-35
Annealing ^b	50-72°C	10-30 s	
Extension ^c	72°C	5-15 s/kb	
Final extension	72°C	5-10 min	1

a: It is essential to completely denature the template DNA at the beginning of PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-2 min denaturation at 95°C is sufficient.

b: A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 2 min.

c: The recommended extension step is 5-10 s/kb at 72° for plasmid template ,10-15 s/kb for routine genomic DNA, 15-30 s/kb for complex template.

Note

For your safty and health,please wear safety glasses, gloves, or protective clothing.

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