NGS Multiplex PCR Master MixII, 2X

NGS Multiplex PCR Master MixII is a PCR Master Mix designed for targeted amplification in Next-Generation Sequencing (NGS). This product contains antibody-modified mutant Hotstart HIFI DNA polymerase, combined with GDSBio's specially formulated NGS PCR Buffer. Primers with different GC contents can use a uniform annealing temperature, enabling ultra-low bias PCR amplification, up to 10,000x multiplex PCR, and can tolerate PCR inhibitors such as SDS, guanidine salts, and heparin. The fidelity performance is more than 100 times that of Taq, and it can be used for targeted NGS sequencing of viral, microbial, tumor/heritable DNA.

Cat. No.	Contents	Storage Conditions
NM2001	NGS Multiplex PCR Master MixII, 2X, 40 rxns	Store unopened at −15°C to −25°C until the expiration date on the label. After opening, the master mix may be stored at −15°C to −25°C until the expiration date on the label, or at 4°C for up to 30 days. The GC Enhancer must be kept at −15°C to −25°C.
	• NGS Multiplex PCR Master MixII, 2X (1 × 1 mL)	
NM2002	NGS Multiplex PCR Master MixII, 2X, 400 rxns	
	• NGS Multiplex PCR Master MixII, 2X (10 × 1 mL)	
NM2003	NGS Multiplex PCR Master MixII, 2X, 2000 rxns	
	• NGS Multiplex PCR Master MixII, 2X (5 × 10 mL)	

Protocol

Note: Before setting up the PCR reactions, prepare a Primer Mix with 0.5 μM of each primer.

Prepare the PCR Reaction Mix

1. Allow all reagents to thaw completely. Mix gently by inverting the tube. Spin briefly. Put all reagents on ice.
2. Using a 96-well optical reaction plate, add the following to one well per sample:

Component	Volume or Mass	Final Concentration
NGS Multiplex PCR Master MixII, 2X	25 μL	1X
Primer Mix (0.5 μM each)	5 μL	50 nM each primer[1]
Template DNA	0.1–0.2 μg	2–4 ng/μL
GC Enhancer	0 or 6 μL [2]	0 or 12%
Nuclease-free water	Adjust to 50 μL	n/a

[1] The final concentration of each primer in a typical PCR is between 0.05-0.4 μM. In most cases, a final concentration of 0.15 μM gives satisfactory results. Increasing the primer concentration up to 0.4 μM may increase the yield.

[2] Use GC Enhancer only when high GC content targets cannot be amplified under standard conditions.

3. Seal the reaction plate with Clear Adhesive Film.

Amplify DNA for Analysis

Choose an amplification protocol based on your analysis method

Amplify for Multiplex PCR

1. Configure the run method as outlined in your instrument's user manual. Use the following parameters:

Step	**Time**	**Temperature (°C)**
Hold	3 min	95
35 Cycles	20 sec	95
	90 sec	60 [1]
	90 sec	72
Hold	5 min	72
Hold	∞	4

[1] Primers with different GC contents can use a uniform annealing temperature, and a lower annealing temperature can achieve high sensitivity.

2. Mix well and briefly spin the reaction plate.
3. Load the reaction plate into the instrument, and start the run. See your instrument's user manual for detailed instructions on how to load and run the plate.
4. Analyze the data according to the instructions in the user manual for your gel electrophoresis instrument.