NGS Multiplex PCR Master MixIII, 2X

Description

NGS Multiplex PCR Master MixIII is a PCR Master Mix designed for NGS unbiased target amplification. By optimizing the enzyme and buffer, this product significantly improves the success rate and consistency of amplification efficiency for GC/AT rich amplification. Primers with different GC content can use a uniform annealing temperature of 55°C to achieve unbiased PCR amplification, up to 10,000x multiplex PCR, which can be used for viral, microbial, and tumor/hereditary DNA target NGS sequencing. It is also suitable for long-fragment multiplex PCR, with the longest fragment reaching up to 20kb, meeting the long-fragment multiple amplification needs for third-generation sequencing.

Components

Cat. No.	Contents	Storage Conditions
NM3001	NGS Multiplex PCR Master MixIII, 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.
	• NGS Multiplex PCR Master MixIII, 2X (1 × 1 mL)	
NM3002	NGS Multiplex PCR Master MixIII, 2X, 400 rxns	
	• NGS Multiplex PCR Master MixIII, 2X (1 × 10 mL)	
NM3003	NGS Multiplex PCR Master MixIII, 2X, 2000 rxns	
	• NGS Multiplex PCR Master MixIII, 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 MixIII, 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
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.

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	2 min	95
35 Cycles	30 sec	95
	60 sec	55 [1]
	60 sec/kb	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.