NGS Multiplex PCR Master Mix, 2X

The NGS Multiplex PCR Master Mix contains all of the components for NGS multiplex PCR (except for primers and templates) in a single tube, including antibody-modified Hotstart DNA Polymerase. With the low error rates and high specificity, the PCR products can be used for NGS sequencing. The mix also includes the GC Enhancer for difficult-to-amplify templates, especially for templates with high GC content.

Cat. No.	Contents	Storage Conditions
NM1001	NGS Multiplex PCR Master Mix, 2X, 40 reactions	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 Mix, 2X (1 × 1 mL)	
	• GC Enhancer (1 × 0.25 mL)	
NM1002	NGS Multiplex PCR Master Mix, 2X, 400 reactions	
	• NGS Multiplex PCR Master Mix, 2X (10 × 1 mL)	
	• GC Enhancer (2 × 1 mL)	
NM1003	NGS Multiplex PCR Master Mix, 2X, 2000 reactions	
	• NGS Multiplex PCR Master Mix, 2X (5 × 10 mL)	
	• GC Enhancer (1 × 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 Mix, 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 MicroAmp Clear Adhesive Film.

Amplify DNA for Analysis

Choose an amplification protocol based on your analysis method

Amplification program for single-round 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	50 [1]
	90 sec	72
Hold	5 min	72
Hold	∞	4

[1] This product needs lower annealing temperature, no more than 55℃.

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.

Technical Resources and Support

For the latest technical resources and support information for all locations, please refer to our website at www.gdsbio.com