NGS Multiplex PCR Master Mix II, 2X

The NGS Multiplex PCR Master Mix II contains all of the components for NGS multiplex PCR (except for primers and templates) in a single tube, including chemically modified ultra-high fidelity DNA Polymerase, which has 100X amplification fidelity of Taq DNA Polymerase. The PCR products have an ultra-low error rates, and is very suitable for the detection of tumor ctDNA and MRD.

Cat. No.	Contents	Storage Conditions
NM2001	NGS Multiplex PCR Master Mix II, 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 II, 2X (1 × 1 mL)	
	• GC Enhancer (1 × 0.25 mL)	
NM2002	NGS Multiplex PCR Master Mix II, 2X, 400 reactions	
	• NGS Multiplex PCR Master Mix II, 2X (10 × 1 mL)	
	• GC Enhancer (2 × 1 mL)	
NM2003	NGS Multiplex PCR Master Mix II, 2X, 2000 reactions	
	• NGS Multiplex PCR Master Mix II, 2X (5 × 10 mL)	
	• GC Enhancer (1 × 10 mL)	

Protocol

Note: Before setting up the PCR reactions, prepare a Primer Mix II 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 II, 2X	25 μL	1X
Primer Mix II(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 analysis by agarose gel electrophoresis

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

Three-step reaction system:

Step	Time	Temperature (°C)
Hold	1 min	98
25-35 Cycles	15 sec	98
	30 sec	58
	30 sec	72
Hold	2 min	72
Hold	∞	4

Two-step reaction system(Tm>60℃):

Step	Time	Temperature (°C)
Hold	1 min	98
25-35 Cycles	15 sec	98
	60 sec	60
Hold	2 min	72
Hold	∞	4

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.

Amplify for CfDNA Sequencing

Component	Volume
NGS Multiplex PCR Master Mix II, 2X	12.5 μL
Primer Mix II	2 μL
cfDNA	X μL
Nuclease-free water	To 25 μL

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

Three-step reaction system:

Step	Time	Temperature (°C)
Hold	1 min	98
25-35 Cycles	15 sec	98
	30 sec	58
	30 sec	72
Hold	2 min	72
Hold	∞	4

Two-step reaction system(Tm>60℃):

Step	Time	Temperature (°C)
Hold	1 min	98
25-35 Cycles	15 sec	98

	60 sec	60
Hold	2 min	72
Hold	∞	4

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.

Technical Resources and Support

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