YTA Multiplex PCR MasterMix 2X with UDG

2X Multiplex PCR Mastermix +UDG	100 µl

For research use only
Cat No: YT1557
Size: 1 ml (40 rxn)
Store at –20°C or at 4°C for up to 30 days.

YTA Multiplex PCR Master Mix with UDG is used to prevent non-specific PCR amplification and contamination which is containing of all components (except primers and templates) required for multiplex PCR reactions. DUTP /UDG anti-pollution system is introduced in the Mix, and UDG enzyme can rapidly degrade the pollutants containing U at room temperature. Hotstart Taq DNA Polymerase's superior performance in combination with an optimized buffer system increases the specificity of the reaction.

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:

1. Preparation of reaction solution
Add the following reagents to the proper thermal cycler reaction tube or plate on ice:

Component	Volume	Final concentration
2X Multiplex PCR Master Mix with UDG	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
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) 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:

Stage	Time	Temprature
Hold	3 min	95℃
35 Cycles	30 sec	95℃
	90 sec	58 ℃
	90 sec	72 ℃
Hold	5 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

Componenet	Volume
2X Multiplex PCR Mastermix +UDG	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:

Stage	Time	Temprature
Hold	3 min	95℃
25-35 Cycles	30 sec	95℃
	90 sec	58 ℃
	90 sec	72 ℃
Hold	5 min	60 ℃
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.