applied biosystems
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Platinum® Multiplex PCR Master Mix, 2×

Insert PN 4463721 Rev. B

Part Number	Part	Storage Conditions
4464268†	Platinum® Multiplex PCR Master Mix, 2×, 50 reaction kit	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.
	• Platinum® Multiplex PCR Master Mix, 2× (1 tube, 1.25 mL)	
	• GC Enhancer (1 tube, 0.3 mL)	
4464269†	Platinum® Multiplex PCR Master Mix, 2×, 250 reaction kit	
	• Platinum® Multiplex PCR Master Mix, 2× (5 tubes, 1.25 mL each)	
	• GC Enhancer (1 tube, 1.25 mL)	
4464270†	Platinum® Multiplex PCR Master Mix, 2×, 2000 reaction kit	
	• Platinum® Multiplex PCR Master Mix, 2× (5 bottles, 10 mL each)	
	• GC Enhancer (1bottle, 10 mL)	

† Only kits are available for direct purchase by customers. Individual components are listed below each kit.

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Platinum® Multiplex PCR Master Mix Protocol* (PN 4463722). For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Protocol

Note: Before setting up the PCR reactions, prepare a 10X 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 (PN 4306737), add the following to one well per sample:

Component	Volume or Mass	Final Concentration
Platinum® Multiplex PCR Master Mix, 2×	25 µL	1 ×
10X Primer mix (0.5 µM each)	5 µL	50 nM each primer
Template DNA	0.1–0.2 µg	2–4 ng/µL
GC Enhancer	0 or 6 µL†	0 or 12%
Nuclease-free water	Adjust to 50 µL	n/a

† 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: (PN 4306311).

Amplify DNA for analysis

Choose an amplification protocol based on your analysis method

Amplify for analysis by agarose gel electrophoresis

Note: For gel electrophoresis, we recommend using the Invitrogen E-Gel® 4% High-Resolution Agarose gels (PN G5018-04) or E-Gel® 48 4% Agarose gels (PN G8008-04). Load 10 µL of the PCR product/lane. Run a 50 bp DNA ladder in parallel.

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
	90 sec	60
	1 min/kb of the largest amplicon	72
Hold	10 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 analysis by capillary electrophoresis

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
28 Cycles	30 sec	95
	90 sec	60
	1 min/kb of the largest amplicon	72
Hold	30 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.
4. Analyze the data according to the instructions in the user manual for your capillary electrophoresis instrument.

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