applied biosystems

Platinum™ Multiplex PCR Master Mix

USER GUIDE

Catalog Numbers 4464268, 4464269, 4464270

Publication Number 4463722

Revision B

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Manufacturer: Life Technologies Corporation | 2130 Woodward Street | Austin, TX 78744

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history : Pub. No. 4463722

Revision	Date	Description
A	April 2011	Base document
B	13 April 2018	Removal of discontinued SKUs and brand update.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

TRADEMARKS: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

Contents

Product information

Product description

End-point multiplex PCR enables simultaneous amplification of many targets in a single tube using multiple pairs of primers. Multiplex PCR has been applied in research, forensic, and diagnostic laboratories where simultaneous analysis of multiple markers is required.

Examples of experiments performed using multiplex PCR include:

- Genotyping (deletions, mutations, and high-throughput SNP profiling)
- Detection of pathogens or genetically modified organisms
- Microsatellites analysis
- Forensic analysis (human identification and paternity testing)
- Quantitative and reverse transcription PCR assays for gene expression

The Platinum™ Multiplex PCR Master Mix contains all of the components for multiplex end-point PCR (except for primers and templates) in a single tube, including Platinum™ *Taq* DNA Polymerase. The kit also includes the GC Enhancer for difficult-to-amplify templates, especially for templates with high GC content.

This master mix is optimized for simultaneous amplification of multiple targets ranging in size from 50–2500 bp from a DNA template with a wide range of GC content.

The PCR products can be analyzed using:

- Agarose gel electrophoresis
- Capillary electrophoresis
- Agilent™ Bioanalyzer™ Instrument

Contents and storage

Component	Cat. No. 4464268 (50 reactions)	Cat. No. 4464269 (250 reactions)	Cat. No. 4464270 (2000 reactions)	Storage[1]
Platinum™ Multiplex PCR Master Mix, 2X	1 × 1.25 mL	5 × 1.25 mL	5 × 10 mL	–25 to –15°C until expiration, or at 4°C for up to 30 days
GC Enhancer	1 × 0.3 mL	1 × 1.25 mL	1 × 10 mL	–25 to –15°C

[1] See label for expiration date.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier. For additional product recommendations, see Appendix D, "Ordering information".

Item	Source
Equipment	
Thermal cycler	MLS
Centrifuge with plate adapter	MLS
Microcentrifuge	MLS
Adjustable pipettors	MLS
Laboratory mixer (vortex or equivalent)	MLS
Tubes, plates, and other consumables	
Optical reaction plates and adhesive covers	thermofisher.com/plastics
Pipette tips with filter plugs	thermofisher.com/pipettetips
1.5-mL microcentrifuge tubes	thermofisher.com/plastics
Polypropylene tubes	MLS
Disposable gloves	MLS
Electrophoresis apparatus	MLS
Agarose or pre-cast gels (1% to 4%)	MLS
Reagents	
Nuclease-free Water (not DEPC-Treated)	AM9930
TE, pH 8.0	AM9849

Workflow

"Prepare the PCR Reaction Mix" on page 7

▼

"Prepare the PCR reaction plate" on page 8

▼

"Set up and run the PCR instrument" on page 9

Methods

Before starting

- Design primers for multiplex PCR. See Appendix B, "Design, verify and synthesize the primers" for details.
- Verify the primers produce a single band of the correct size using singleplex PCR. See "Guidelines for primer verification" on page 15 for details.

Prepare primer mix

1. Combine all primers for the multiplex PCR reaction in one tube, and adjust the final concentration to 0.5 µM per primer using 0.1× TE (10 mM Tris-HCl, 0.1 mM EDTA) buffer.
2. Dispense the primer mix into aliquots. Store at –20°C for up to one year or 4°C for up to two months.

Preparations for PCR

- Before you begin PCR, review "PCR good laboratory practices" on page 16.
- Select an instrument and reaction plate
- Calculate the number of reactions needed to perform each assay, including no-template control (NTC) reactions. Add at least one extra reaction for every 10 required reactions to account for volume loss from pipetting. For example, for a 96-well plate, prepare enough volume for approximately 110 reactions.
- Multiple PCR reactions can be run on one reaction plate, but include controls for each run on the plate.

Prepare the PCR Reaction Mix

1. Thaw the primer mixes, templates, GC Enhancer (optional), and Platinum™ Multiplex PCR Master Mix.
2. Mix the Platinum™ Multiplex PCR Master Mix by gently inverting the tube 10 times, then place on ice.

   IMPORTANT! Avoid bubble formation during mixing.

3. Mix the remaining reagents by inverting each tube a few times, then centrifuge the contents briefly. Place the tubes on ice.

4. Combine the following components for the number of reactions required, plus an additional 10% of volume.

Component	Volume per 50-µL reaction	Final concentration
Platinum™ Multiplex PCR Master Mix, 2X	25 µL	1X
Primer mix (0.5 µM each primer)	5–10 µL [1]	50–100 nM each primer
(*Optional*) GC Enhancer	0–10 µL [2]	0–20%
Nuclease-free Water	50 µL minus template DNA volume[3]	—

[1] Use 10 µL of primer mix (100 nM final concentration of each primer) when less than 0.1 µg of DNA is used.

[2] For targets with 65 to 75% GC, use 6 µL of GC Enhancer in a 50-µL reaction. For targets with >75% GC, start with 10 µL in a 50-µL reaction. See "Enhancer concentration" on page 17 for details.

[3] This protocol assumes that identical volumes of template DNA will be used for each reaction.

5. Mix the PCR Reaction Mix by inverting the tube a few times.

6. Centrifuge briefly to bring the PCR Reaction Mix to the bottom of the tube and eliminate air bubbles.

Prepare the PCR reaction plate

1. Dispense appropriate volumes (50 µL minus the template DNA volume) of the PCR Reaction Mix to the wells of a reaction plate or PCR tubes.

2. Add 0.1–0.2 µg of DNA template to the PCR Reaction Mix to bring the final volume to 50 µL.

   Note: Adjust the final reaction volume of each no-template control well or tube to 50 µL with Nuclease-free Water.

3. Seal the reaction plate with optical adhesive film, or cap the tubes.

4. Mix the contents well by inverting the plates or tubes a few times.

   Note: Inverting the plate gives more uniform mixing across the reaction plate than vortexing.

5. Centrifuge briefly to bring the contents to the bottom of the wells or tubes and eliminate air bubbles.

Set up and run the PCR instrument

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions or to run the plate.

1. Select the appropriate amplification protocol based on your analysis method.

   Amplification protocol for analysis by agarose gel electrophoresis or Agilent™ Bioanalyzer™ Instrument.

Step	Cycles	Temperature	Time
Activation	1	95°C	2 min
Denaturation	30–40	95°C	30 s
Annealing		60°C	90 s
Extension		72°C	60 s/kb of largest amplicon
Final extension	1	72°C	10 min
Final hold	1	4°C	Hold

   Amplification protocol for analysis by capillary gel electrophoresis.

Step	Cycles	Temperature	Time
Activation	1	95°C	2 min
Denaturation	25–40	95°C	30 s
Annealing		60°C	90 s
Extension		72°C	60 s/kb of largest amplicon
Final extension	1	60°C	30 min
Final hold	1	4°C	Hold

2. Load the reaction plate or tubes into the PCR instrument, then start the run.

Analyze the PCR products

You may analyze results by agarose gel electrophoresis, capillary gel electrophoresis, or Agilent™ Bioanalyzer™ Instrument. See the instrument documentation for analysis details.

Analyze bands by agarose gel electrophoresis

Choose a gel with the appropriate agarose concentration according to the total number of amplicons, the size difference between adjacent amplicons, and the amplicon size range in each reaction.

1. Unload the reaction plate or tubes after the run is complete.
2. (Optional) Store the plate or tubes at 4°C for up to 72 hours, or at –25 to –15°C for long-term storage.
3. Load and run gels according to the manufacturer's instructions to achieve the maximum separation of adjacent amplicons.
4. Take pictures of the gels on a transilluminator appropriate for the type of DNA visualization dye used in the agarose gel.

A

Troubleshooting

Observation	Possible cause	Recommended action
Excessive amount of primer dimers	Sub-optimal primer design.	Review primer design and composition, and ensure each primer pair gives a single band and no primer dimer in singleplex PCR.
	Cycle number is too high.	Reduce the cycle number in increments of three cycles.
	Primer manufacturing error.	Order primers from a reliable vendor and ensure that a purification process like de-salting and HPLC is used.
	Inaccurate volumes when pipetting.	Calibrate pipettes regularly to ensure the accuracy of volumes.
	Low quality template DNA.	Always use high-quality, purified DNA templates.
Little or no PCR product detected	Template concentration is too low.	Increase the sample input to 0.1–1 µg/ 50 µL reaction.
	Template DNA is damaged or degraded.	Use a larger amount of template DNA or use DNA from a sample that has been processed to minimize shearing and nicking.
	Denaturation time is too short or too long.	Adjust the denature time up or down by 5 seconds increments.
	Denaturation temperature is too low or too high.	Adjust the temperature by 1°C increments. If using a Veriti™ Thermal Cycler, adjust the VeriFlex™ blocks by 2°C increments for up to six different temperatures.
	Annealing/extension time is too short.	Increase the time by 15 second increments.
	Cycle number is too low.	Increase the cycle number by three cycle increments.
	Sub-optimal primer design.	Review primer design and composition.
	Preincubation/activation time is insufficient.	Increase the polymerase activation step by 1 minute increments.
PCR product bands are smeared	Potential secondary products amplified.	Reduce final concentrations of the primers for the select amplicons to 50 nM while maintaining all other at 100 nM.
	Carryover contamination.	Dispose of reagents, make fresh reagents, then repeat the PCR.
	Denaturation time is too short or too long.	Adjust the time up or down by 5 seconds increments.
	Denaturation temperature is too low.	Increase the temperature by 1°C increments.

Observation	Possible cause	Recommended action
PCR product bands are smeared	Annealing/extension time is too long.	Shorten the time by 15 second increments.
	Cycle number is too high.	Shorten the cycle number by three cycle increments.
	Template DNA is degraded.	Test a new aliquot of sample.
	Amount of template DNA is too low.	Increase the template DNA input to 0.1–1 µg/50 µL reaction.

B

Design, verify and synthesize the primers

Select an amplicon and design the primers

IMPORTANT! If GC-rich targets are unavoidable when selecting your amplicon site and primers, keep the amplicon sizes shorter than 300 bp. Avoid simultaneous amplification in a single reaction of targets with over 70% GC and those with less than 25% GC when mixing primers. GC Enhancer is included as an optional PCR additive for targets with greater than 65% GC content.

Use the Primer Express™ or Primer3Plus software packages (or equivalent) for selecting your amplicon site and designing the primers.

- Primer Express™ Software—Refer to the *Primer Express™ Software Version 3.0 Getting Started Guide* and *Software Help* for guidance on using the software.
- **Primer3Plus**—Freeware provided at **www.bioinformatics.nl/primer3plus**. See "Design primers with Primer3Plus freeware" on page 13 for additional guidance on using the software.

  Note: Primer3Plus provides a Basic Local Alignment Search Tool (BLAST) algorithm, which is useful for comparing sequence information. Other software options do not provide this algorithm.

Design primers with Primer3Plus freeware

1. In the **Main** tab, paste your sequence ID and the source sequence.
2. In the **General Settings** tab, define the variables according to the following table.

Variable	Definition
Primer size	21–30 nt
Primer T_m	59–65°C
Primer GC%	40–60%
Concentration of monovalent cations	50 mM
Annealing oligo concentration	50 nM
Mispriming/Repeat Library	Based on your assay
Remaining variables	Default or user-defined

3. In the **Advanced Settings** tab, define the product size.
   - For GC-rich sites, amplicon size should be <300 bp.
   - Ensure there is sufficient size differentiation between adjacent amplicons for adequate separation.
     – If performing analysis by E-Gel™ High-ReSolution Agarose Gels, 4% ensure that amplicons meet the minimum size difference provided in the following table.

Size of the adjacent amplicons	Minimum difference
50–100 bp	10 bp
100–200 bp	20 bp
200–400 bp	40 bp
400–700 bp	50 bp
700–900 bp	100 bp
900–2500 bp	500 bp

     – If performing analysis by E-Gel™ General Purpose Agarose Gels use a gel with the appropriate agarose content based on the estimated resolution provided in the following table.

Agarose content	Run length	Resolution
0.8%	5.8 cm	800 bp–3 kb
1.2%		100 bp–3 kb
2%		100 bp–2 kb

Verify primer compatibility for multiplex PCR

Verify the designed primers for their multiplex PCR compatibility using the UCSC In-Silico PCR utility at **genome.ucsc.edu/cgi-bin/hgPcr**

1. Access the UCSC In-Silico PCR utility from your computer.
2. Enter the sequences of forward and reverse primers, then click **Submit**.
3. Verify that the requirements in the following list are met.
   - The PCR product is unique.
   - The size of the product differentiates it from adjacent amplicons.
   - The T_m for each primer is between 59–65°C.
4. Copy the sequence of the product, then click **BLAT**.
5. Paste the sequence from the In-Silico PCR utility into the text box in the Blat window, then click **Submit**.
6. Click **Details** for each retrieved fragment to ensure there is no homology in the primer targeting sites.

Guidelines for multiplex PCR primers

- Synthesize primers with a reliable oligo vendor and dissolve them in 0.1× TE buffer (10 mM Tris-HCl, 0.1 mM EDTA: pH 8 for regular oligos, and pH 7 for fluorescent labeled oligos).
- De-salted primers are generally pure enough for multiplex PCR.
- Verify the concentration of each primer by UV spectrophotometer before use.
- Store aliquots of primers at –20°C. Avoid repeated freeze-thaw cycles.

Guidelines for primer verification

- Before proceeding to multiplex PCR, ensure that singleplex PCR with the primers results in a single band of the correct size if using template DNA that is homozygous for the amplified target.
- If a primer pair produces multiple bands from homozygous template DNA, redesign the primers by changing the location of the targeting site on the genome until a single band is produced.

C

Guidelines for PCR assays

PCR good laboratory practices

When preparing samples for PCR amplification:

- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Do not bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Centrifuge tubes before opening. Avoid splashing or spraying PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNA*Zap*™ PCR DNA Degradation Solutions (Cat. No. AM9890).

Adjust thermal cycler conditions

Polymerase activation

For general PCR runs, a pre-PCR activation setup of 95°C for 2 minutes is recommended.

Denaturation conditions

- In the early cycles, make sure that your DNA template is completely denatured.
- Do not exceed a denaturation temperature of 95 to 96°C.
- A denaturation period of 30 seconds is adequate when using a Veriti™ Thermal Cycler with a calculated in-tube temperature. Some models of thermal cyclers may require longer denaturation times.

Annealing conditions

- For increased product specificity, use annealing temperatures higher than 45°C.
- Determine the optimum annealing temperature by testing at increments of 5 or fewer degrees Celsius until the maximum specificity is reached.
- Narrow the range of annealing temperatures to test by calculating primer melting temperatures (T_m). For an online tool to determine T_m, go to **thermofisher.com/tmcalculator**.
- A 30 second annealing time is adequate when using a Veriti™ Thermal Cycler with a calculated in-tube temperature. Other thermal cycler models may require longer annealing times.

Extension conditions

- The length of the target sequence affects the required extension time. Longer targets require increased extension times. In general, allow an extension time of approximately 60 seconds per 1000 bases at 72°C.
- As the amount of DNA increases, the number of DNA polymerase molecules may become limiting. Compensate for this limitation by increasing the extension time in later cycles.

Optimize the PCR conditions

Template concentration

- The DNA segment to be amplified from the template can be up to 2.5 kb long, although 100 to 1500 bases are more typical and easier to amplify.
- Start with enough copies of the template to obtain a signal after 25 to 30 cycles. More than 30 ng (10^4 copies) but less than 1 µg of human genomic DNA per 50-µL reaction is the recommended range.
- If the target DNA concentration is low, you may need more than 35 cycles to produce sufficient product for analysis, however, this may increase the likelihood of primer dimers.

Enhancer concentration

The GC Enhancer helps amplify challenging amplicons, including amplicons that are GC-rich, have GC-repeats, or generate nonspecific products. GC enhancer is generally not needed if the GC contents of the targets are between 25 and 70%.

- In a 50-µL reaction, for targets with 65 to 75% GC, start with 6 µL.
- In a 50-µL reaction, for targets with >75% GC, start with 10 µL.

In general, if increased specificity is required, add 1 to 2 µL per 50-µL reaction.

The GC Enhancer may reduce nonspecific amplification and improve the yield of specific products. However, excessive use of the GC Enhancer may reduce yield, particularly for non-GC-rich amplicons.

D Ordering information

Equipment

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Product	Cat. No.
Thermal cycler	
Veriti™ 60-Well Thermal Cycler	4384638
Veriti™ 96-Well *Fast* Thermal Cycler	4375305
Veriti™ 96-Well Thermal Cycler, with 0.2-mL sample wells	4375786
Electrophoresis apparatus	
E-Gel™ Power Snap Electrophoresis Device	G8100
Mother E-Base™ Device	EBM03

Consumables

Product	Cat. No.
Reagents	
Nuclease-free Water (not DEPC-Treated)	AM9930
TE, pH 8.0	AM9849
BlueJuice™ Gel Loading Buffer (10X)	10816015
E-Gel™ 50 bp DNA Ladder	10488099
E-Gel™ Low Range Quantitative DNA Ladder	12373031
(Optional) RNase Inhibitor (Cloned; 40 U/µL)	AM2684
Tubes and caps	
MicroAmp™ 8-Tube Strip, 0.2 mL	N8010580
MicroAmp™ 8-Cap Strip, clear	N8010535

Product	Cat. No.
96-Well Plates and adhesive film	
MicroAmp™ Optical 96-Well Reaction Plate	N8010560
MicroAmp™ Clear Adhesive Film	4306311
PCR accessories	
MicroAmp™ Optical Film Compression Pad	4312639
MicroAmp™ 96-Well Tray/Retainer Set	403081
MicroAmp™ 96-Well Base	N8010531
MicroAmp™ Splash-Free 96-Well Base	4312063
Pre-cast agarose gels	
E-Gel™ High-ReSolution Agarose Gels, 4%	G501804
E-Gel™ *48* Agarose Gels, 4%	G800804

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.